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# CONSEQUENCES OF MULTIPLE SCLEROSIS FOR PATIENTS IN SWEDEN

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# Consequences of Multiple Sclerosis for Patients in Sweden

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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# ABSTRACT

This study aimed to consider consequences of multiple sclerosis for patients in Sweden throughout various stages of the life course. The thesis was separated into four constituent papers, which dealt with different aspects of the disease, its symptoms, and implications at different life stages.

The first study considers life expectancy, one of the most crucial aspects concerning the implications of the consequences of a chronic disease. The paper found MS patients had a hazard ratio (HR) for mortality of 2.92 (95% confidence interval (CI) 2.86-2.99)) at any given age relative to a group of non-MS comparators when the entire study period from 1968 to 2012 was analysed. When trends were considered, however, it was shown that the improvement to survival for MS patients had been considerable, and dropped from an HR of 6.52 (95% CI 5.79–7.34) when considering the earliest time period (1968 to1980), down to an HR of 2.08 (95% CI 1.95–2.22) for the most recent time period (2001 to 2012). Cause specific mortality also improved over time for MS patients, with mortality beginning to more closely reflect mortality trends for the general population. The largest excess mortality for MS patients came from respiratory and infectious diseases. Cardiovascular disease was the leading cause of death for both the MS and non-MS cohorts.

Alongside issues pertaining to life expectancy, how patients are affected by their symptoms is an important consideration when answering questions about consequences of MS. Pain has been noted as a particularly distressing symptom by MS patients, and previous studies have indicated it is likely MS patients experience pain to a greater degree than the general population. As far as we are aware, however, there have been very few studies making direct comparisons of pain between MS patients and non-MS comparators with regard to pain, perhaps due to difficulties in drawing direct comparisons. In order to attempt objectivity and a fair comparison across MS and non-MS subjects, the second and third studies utilized the prescribed drugs register (PDR) of Sweden in order to ascertain when prescriptions for pain relief had been collected. An excess of pain relief prescriptions would imply an excess of pain among MS patients. Information on pain type can also be extracted using this method through the anatomical therapeutic code (ATC) entered into the PDR. Study two was able to provide evidence supporting the hypothesized increased risk of pain among MS patients, and demonstrated MS patients had an HR of 2.52 (95% CI 2.38-2.66) for overall pain prescription. It was additionally shown that this increased risk of pain was primarily driven by increased likelihood of neuropathic pain. The HR for MS patients being prescribed these treatments relative to their non-MS comparators was 5.73 (95% CI 5.07-6.47). MS patients were also at marginally increased risk of anti-migraine preparation prescriptions, however no increased risk of prescriptions for the treatment of musculoskeletal pain was detected.

Study three followed on from study two, which considered pain relief prescription, and included the same definition of the outcome. However, the study aimed primarily to consider the effect of genotype on MS and pain phenotype. Past murine studies have indicated that the major histocompatibility complex (MHC) is associated with pain-like behavior when considering peripheral nerve injury, however the same association was not observed when considering injury to the central nervous system (CNS), which more closely mimics the nervous system injuries seen in MS patients due to demyelination. Past research has identified that the DQB1*0302 class II HLA genes are associated with neuropathic pain presentation in individuals undergoing surgery for inguinal hernia, or for spinal disc herniation. As far as we are aware, the role of this allele in pain presentation, and whether it is differential by MS status has not been previously studied. A modest increased risk of pain for non-MS carriers of the DQB1*0302 allele was found, with an odds ratio (OR) of 1.18 (95% CI 1.03-1.35), however no increased risk was identified for MS patients (OR 1.02, (95% CI 0.85-1.24)), mimicking the results found in murine studies.

Given that the average age at diagnosis is childbearing age for women, and that the majority of patients are women, issues surrounding MS and pregnancy were important to consider when answering questions of consequences of MS. Paper four assessed whether exposure to interferon –beta during pregnancy influenced intrauterine growth, by considering its effect on birth weight, height, and head circumference. This was an international study comprised of data from both Sweden and Finland. The study, which used prescribed medication to identify pre-natal exposure, and additionally the MS register within Sweden, concluded that exposure to interferon-beta did not seem to be associated with intrauterine growth in either Sweden or Finland.

# LIST OF SCIENTIFIC PAPERS

I. Burkill S, Montgomery S, Hajiebrahimi M, Hillert J, Olsson T, Bahmanyar S (2017), Mortality trends for multiple sclerosis patients in Sweden between 1968 and 2012, *Neurology,* 89 (6): 555-562

II. Burkill S, Montgomery S, Kockum I, Piehl F, Stridh P, Hillert J, Alfredsson L, Olsson T, Bahmanyar S (2019), The association between multiple sclerosis and pain medications, *Pain,* 160 (2): 424-432

III. Burkill S, Smith KA, Stridh P, Kockum I, Lindahl H, Alfredsson L, Olsson T, Piehl F, Montgomery S, Bahmanyar S, The DQB1*0302 genotype and treatment for pain in people with and without Multiple Sclerosis, Under review

IV. Burkill S, Vattulainen P, Geissbuehler Y, Sabido M, Popescu C, Suzart-Woischnik K, Hillert J, Artama M, Verkkoniemi-Ahola A, Myhr KM, Cnattingius S, Korhonen P, Montgomery S, Bahmanyar S, The association between exposure to interferon-beta during pregnancy and birth measurements in offspring of women with multiple sclerosis, Under review

**List of papers during the PhD period not included as constituent papers, in the field of neurological disease**

Iacobaeus E, **Burkill S**, Bahmanyar S, Hakim R, Byström C, Fored M, Olsson T, Brundin L, Montgomery S (2018), The national incidence of PML in Sweden, 1988-2013, *Neurology*, 90 (6), pp498-508, DOI: 10.1212/WNL.0000000000004926

Sundholm A, **Burkill S**, Bahmanyar S, Nilsson Remahl AIM (2018), Improving identification of Idiopathic Intracranial Hypertension patients in Swedish patient-registry, *Acta Neurologica Scandinavica*, 137(3) pp.341-346, DOI: 10.1111/ane.12876

Montgomery S, Hiyoshi A, **Burkill S**, Alfredsson L, Bahmanyar S, Olsson T (2017), Concussion in adolescence and risk of multiple sclerosis, *Annals of Neurology*, 82 (4), pp554-561, DOI: 10.1002/ana.25036

Sundholm A, **Burkill S**, Sveinsson O, Piehl F, Bahmanyar S, Nilsson Remahl A (2017), Population-based incidence and clinical characteristics of idiopathic intracranial hypertension, *Acta Neurologica Scandinavica*, 136 (5), pp 427-433, DOI: 10.1111/ane.12742

Montgomery S, Hajiebrahimi MH, **Burkill S**, Hillert J, Olsson T, Bahmanyar S (2016), Multiple sclerosis and risk of young-adult onset Hodgkin's lymphoma, *Neurology Neuroimmunology & Neuroinflammation*, 3 (3): e227. doi:10.1212/NXI.0000000000000227: 2332-7812

Brenner P, **Burkill S**, Jokinen J, Hillert J, Bahmanyar S, Montgomery S (2016), Multiple sclerosis and risk of attempted and completed suicide- a cohort study. *European Journal of Neurology*. 23(8) p1329-1336. doi: 10.1111/ene.13029

# Contents

# INTRODUCTION

Multiple sclerosis (MS) is the leading non-traumatic cause of disability among young people[1-4], and yet the effect it has on the lives of people living with the disease is incompletely described. The disease is chronic in nature, and progresses over may years, making it difficult to study using conventional methods such as questionnaires and follow-up studies, due to issues such as attrition. It can also be complicated studying personal experiences and outcomes of chronic diseases using subjective measures which require recall from patients and other study participants.

The use of large scale register data allows for a unique opportunity in investigating the long term consequences of MS. Within Sweden, administrative records of health, mortality, and other important outcomes and exposures have been kept for several decades, all of which can be linked using Swedish personal identification numbers[5], giving the opportunity to look into consequences of MS without the concerns relating to attrition and response bias[6]. Records are available for the entire population of residents within Sweden, allowing for matched cohort studies with follow-up times that can span for decades, which has the potential to elucidate suspected associations which had previously been limited to anecdotal evidence taken from patient and clinician experience, or from smaller scale studies with shorter study times.

MS patients can be differently affected by their disease depending on their stage of life[7]. This thesis attempts to take what we considered to be important stages during the life course and the disease progression, and study how patients were affected using primarily register data. The ability to consider 45 years of follow-up in one of our studies enabled us to investigate life expectancy and mortality rates overall, and by specific causes of death, with considerably more power and information than has been available for previous research into this area. Using pain prescriptions as an objective proxy for a pain diagnosis, we were able to study to what extent the hypothesised increased risk of pain for MS patients was found in our data. Genetic susceptibility to pain according to MS status was studied through register data, coupled with genetic information collected from the blood samples of MS and matched comparators. Pregnancy outcomes in the form of birth measurements again taken from registers allowed us to consider how infants exposed to MS treatment during pregnancy are affected, relative to those born to mothers

who decide to discontinue treatment. This removes the effect of recall bias, and allows for a more objective approach into how infants may be affected by treatment exposure, a question of primary concern to women with MS who decide to become pregnant. This can be particularly pertinent because many individuals are diagnosed with MS at an age which coincides with decisions to become a parent. Our hope is that all four studies will be beneficial and provide insight into the consequences of MS for patients in Sweden at different points in a patient's life.

# 1. BACKGROUND

## 1.1. DEFINITION AND BACKGROUND OF MS

MS is an autoimmune disease which leads to demyelination and subsequent damage to neurons [8-12]. Myelin is a lipoprotein produced by oligodentrocyte cells, which enables fast, saltatory impulse propagation[13]. Damage to myelin has importance for the functioning of the CNS, and can lead to symptoms including visual impairment, muscle weakness, and areas of numbness, symptoms which are often present in MS patients[14].

The disease is characterised by the formation of focal demyelinated plaques in the white matter tissue of the central nervous system (CNS)[15]. In the early, usually relapsing stages of the disease (see section 1.3), MS is mainly characterised as a disease of the white matter, however in the later, progressive disease stages, cortical demyelination and injury due to inflammation of all white and grey matter can be observed[16].

## 1.2. CLINICAL DIAGNOSIS

Diagnosing MS can be difficult, and usually relies on ruling out other possible causes of symptoms. In order for a diagnosis of MS to be considered accurate, there should be evidence of injury to the CNS from demyelination due to inflammation, often in the form of a lesion[17]. Oligocolonal bands, proteins which indicate inflammation of the CNS, can also point to an MS diagnosis[18]. The McDonald criteria, established in 2001 as a tool for MS diagnosis based primarily on the concept of evidence of disseminated lesions in time and space[19] remains the gold standard, objective measurement for determining the presence of MS in an individual. Further revisions to the criteria have been proposed since its initial implementation, including simplifications to the use of imaging which

mean dissemination of injury to the CNS in space and time now have the potential to be ascertained through a single scan[20, 21].

The methods available to provide evidence for MS during the diagnostic process have changed over time, in particular with the development of, and improvements to, magnetic resonance imaging (MRI) techniques[22]. Prior to the development of detailed MRIs, examination of cerebrospinal fluid (CSF) for the purposes of ascertaining whether levels of immunoglobulin production were increased was one of the fundamental diagnostic tests for MS[22]. The alterations and evolution of diagnostic criteria over time can potentially impact the characteristics of those defined as having MS, which makes comparisons over time more complicated. Recent innovations in MRI technology has enabled earlier detection and diagnosis of the disease and earlier possibility to begin disease-modifying treatments is therefore possible. The impact this has had on various aspects of the disease course and implications for patients have historically been difficult to study. The consequences of living with the disease both in recent years, and prior to improved diagnostic techniques, is an important study area which could provide valuable insights into the prognosis of MS for patients, and how this may have changed over time.

### 1.3 SUBTYPES OF MS

MS can be separated into four broad categories: relapsing remitting (RR), secondary progressive (SP), primary progressive (PP), and progressive relapsing (PR)[7]. RR MS is the most common subtype, with approximately 85% of incident diagnoses falling into this category. This subtype is defined by exacerbation of symptoms followed by periods of remission, during which a patient may have no or reduced symptoms. For some patients with RR MS, SP MS eventually develops, usually after a number of years[23]. It is believed disease modifying treatments can reduce the risk of SP MS development in some individuals[24]. For patients who develop this subtype, the symptoms continue to worsen with reduced probability of remission between episodes of worsening symptoms. PP MS is a rarer form of MS, which affects approximately 10% of MS patients. This disease subtype is known to be more resistant to conventional MS treatments, and is marked by a worsening of symptoms from the onset of disease with no episodes of remission or relapse[25]. Instead, a gradual worsening of symptoms is experienced by the patient. The last subtype, PR MS is present in approximately 5% of MS patients, and is progressive from disease onset, although debate continues as to whether this subtype is

indeed a distinct clinical phenotype. This group of patients experience worsening of symptoms, with relapses followed by plateaus or phases in which symptoms worsen more gradually. Patients in this subgroup do not experience episodes of remission[26].

# 2. THE EPIDEMIOLOGY OF MS

MS is the most frequently diagnosed demyelinating disease[1], with prevalence highest in North America and Europe, and lowest in sub-Saharan Africa and East Asia[27]. The ratio of diagnoses of MS between females and males is approximately 3:1[28]. The reasons behind the increased likelihood of receiving a diagnosis of MS for women are incompletely understood, however several hypotheses have been proposed including differences in hormone levels, and genetic differences[28]. The ratio appears to have widened in many European countries in recent decades, revealing an increase in MS among women but not among men[29], however this trend was not confirmed using Swedish data, suggesting the underlying mechanisms may be complex and context specific[30].

MS is most commonly diagnosed between the ages of 20 and 50 years, with a mean age of approximately 35 years at diagnosis. Disease course and prognosis has been shown to differ depending on age at diagnosis, although age and cohort effects can be difficult to disentangle[31]. RR MS is often diagnosed at a younger age relative to other MS subtypes, with a mean age at diagnosis of approximately 30 years compared to a mean of 40 years of age for both SP, and PP MS. PR MS is the least common subtype, meaning less is known about the characteristics of individuals affected by this MS type, however there is some evidence to suggest the mean age at diagnosis is approximately 35 years of age, falling between the mean age for RR MS and other subtypes[32].

## 2.1 RISK FACTORS

The causes and risk factors of MS remain incompletely understood, although there are several proposed risk factors, which include genetic predisposition, pattern of exposure to the Epstein-Barr virus, which could be argued to comprise a portion of the hygiene hypothesis, insufficient exposure to sunlight and vitamin D, obesity and smoking[33].

### 2.1.1. Genetic predisposition

Olsson et al. describe a possible 'pathogenetic pathway', in which genetic susceptibility interacts with lifestyle factors, impacting on the functioning of immunity within an individual[33]. A gene-environment interaction is suggested to be a plausible route, with lifestyle therefore recommended as a target for preventative measures given that lifestyle can be altered. Genes within the HLA complex are the strongest genetic indicators of MS risk[34], and are responsible for products which present antigens to CD4 T lymphocytes, and CD8 lymphocytes. In addition, several SNPs (single nucleotide polymorphisms) on the human genome outside the HLA complex have also been identified as possible risk factors for MS[35]. The pathogenetic pathway hypothesis posits that these genes interact with lifestyle and behavioural factors, in such a way that the risk of developing MS among those exposed to both genetic and behavioural factors is higher than the additive effect of exposure to each risk factor, indicating an interactive effect[33]. Such lifestyle factors include smoking, although exposure to nicotine through oral tobacco has been shown to be protective against MS, obesity, and levels of vitamin D exposure.

### 2.1.2. Epstein-Barr virus and vitamin D

Exposure to Epstein-Barr virus is another commonly cited potential causal agent of MS[36]. The relationship between MS and exposure to the virus is difficult to ascertain, and several other infectious agents have been proposed to be associated with increased risk of MS development[33]. The evidence of an association with the Epstein-Barr virus is, however, particularly compelling, in part due to a consensus that the virus is significantly more common amongst MS patients than those without MS[37, 38]. The inflammatory process through which this pathway may function has been under discussion for many years, and is largely based on observations of the nature of demyelination and injury to the CNS observed in MS patients, along with abnormalities found in the cerebrospinal fluid (CSF)[38]. Here the potential interaction between development of MS and insufficient vitamin D exposure has been explored, with one hypothesis suggesting when an individual is exposed to vitamin D, the number of CD8+ T cells increases, which in turn increases the ability of the immune system to control the EBV infection. The implication is those with insufficient vitamin D exposure are left susceptible to the spread of the infection, and are less able to control the virus, and thus become more susceptible to the

development and subsequent diagnosis of MS[39]. Whether or not the relationship between EBV and development of MS is causal or is an association remains to be proven.

Coinciding with the hypothesis that vitamin D exposure may influence the development of MS is the concept that latitude appears to be associated with risk of MS, with incidence and prevalence positively associated with an increase in latitude[40]. This association is, however, not constant across countries. Furthermore, the effect of latitude appears to have decreased in recent years, which suggests latitude may be a confounder rather than a risk factor in itself[40].

### 2.1.3. Hygiene hypothesis and autoimmunity

The hygiene hypothesis theorises that the growing burden in the industrialised world of allergic and autoimmune conditions may relate to reduced exposure to infection[41]. Through improved hygiene, antibiotic use and vaccination programmes, the human immune system may be altered, and become prone to respond inappropriately to harmless substances[42], resulting in hypersensitivity. Hypersensitivity refers to the process by which the immune system responds to non-pathologic antigens, which can take the form of an immune response to allergens and result in an allergic reaction[43], or a response to the body's own tissue, which then results in autoimmunity[44]. MS is believed to primarily be a result of type IV hypersensitivity, which is associated with pathologic T-cells, in particular regarding CD4 T cell responses[45]. It is well established that T cells with specificity for myelin are involved in MS development[46], however other potential mechanisms of hypersensitivity have also been implicated. MS has, for example, been linked to type II hypersensitivity involving the presence of autoantibodies[47]. Autoantibodies are produced by the immune system in response to the presence of a constituent of its own tissues, and can sometimes be detected in serum many years before symptoms manifest[44]. When considering specifically MS, anti-myelin basic protein autoantibodies have been detected in patient serum, proteins which historically were generally believed to be absent from the serum of non-MS individuals[48]. However, recent research has indicated that myelin-reactive autoantibodies can also be found in the serum of individuals without MS[49], making it difficult to ascertain the pathologic role these autoantibodies play. Therefore, the debate about the role of autoantibodies in the pathogenesis of MS is ongoing.

The hygiene hypothesis and its proposed effect on autoimmunity development may hold relevance for understanding the mechanisms behind MS disease course and development. Evidence to suggest MS may be alleviated through infection, and conversely exacerbated through lack of exposure to infection has been documented[50], highlighting a potential association between immune system function and infection. Whether those who have less exposure to infectious agents are at reduced risk of developing MS is, however, yet to be ascertained[51]. Exposure to Epstein-Barr virus could be considered as a specific aspect of the hygiene hypothesis.

### 2.1.4. Obesity

Previous studies have provided evidence of an association between high BMI, and MS susceptibility[52]. Adipose tissue can influence immune system function through secretion of adipokines and cytokines, including leptin, which can influence T-cell activity[53]. This perspective suggests individuals with obesity maintain a low-grade chronic inflammatory state, culminating in increased susceptibility to autoimmune disorders alongside other adverse clinical outcomes[54].

### 2.1.5. Smoking

Smoking has been shown to be a risk factor for MS development regardless of age at exposure, with the increased risk of MS abating a decade after smoking cessation[55]. The mechanism through which smoking influences MS risk is incompletely understood, but a number of theories have been proposed. One possible explanation relates to the cyanide present in inhaled tobacco, which has been shown in animal studies to induce selective demyelination[56]. Cyanide administered in smaller, regular doses, perhaps similar to the doses regular smokers would be exposed to, more successfully produced demyelinative lesions than when one large dose was administered. There is also evidence to suggest cigarette smoke has inflammatory properties such as increasing peripheral leukocyte counts, which could be instigating and exacerbating MS development[57]. Although several theories have been proposed, they have not been conclusively verified, and some have shown nicotine could have a potentially protective effect against the development of autoimmune diseases[58].

## 2.2. TREATMENT OF MS

For treatment naïve MS patients, there are four first line treatments recommended in Sweden: glatiramer acetate (GA), interferon (IFN)-β 1a, and, IFN-β 1b, and dimethyl fumarate (DMF). Corticosteroids are often also used in instances of acute MS relapse[59]. Second line treatments include fingolimod and natalizumab[60]. In addition to the recommended first line treatments, Rituximab is often prescribed off label, meaning there is limited information on the long term safety and efficacy of this treatment for MS patients[61]. When and how to make the transition from first line to second line treatment can be difficult, with no internationally recognised guidelines on when the decision should be taken and how it should be implemented[62]. Immunomodulatory treatment alters the functioning of the immune system, which has the potential to provide improvements to MS patients through reduction in symptoms, and delays in disease progression for in particular RR patients.

### 2.2.1. First Line Treatments

GA, one of the first immunomodulators to be approved for use in RR MS patients, induces the expression of anti-inflammatory cytokines, which can suppress demyelination and reduce the possibility of relapse. IFN-β modulates antigen presentation, and can decrease T-cell production of IFN gamma. This treatment has the possibility to reduce the entry of T-cells into the CNS, thus reducing the probability of damage or injury to the CNS due to T-cell activity[63]. Patients treated with IFN-β commonly experience flu-like symptoms shortly after treatment is administered, which usually subside soon after[64]. Treatment with DMF appears to work through the effects of DMF on the nuclear factor (erythroid derived 2)-like2 (NRF2) pathway, altering NRF2's immune regulatory properties, meaning DMF has the potential to act as a cytoprotecting agent[65].

### 2.2.2. Off label treatment with Rituximab

Rituximab is becoming increasingly common as a treatment for MS. Rituximab is a B-cell-depleting monoclonal antibody acting primarily against CD20[66]. The targeting of CD20, a phosphoprotein expressed on the surface of B-cells, results in B-cell lysis, and subsequently to B-cell peripheral depletion[67]. The effect of rituximab on B-cell depletion reduces inflammatory activity, and therefore has the ability to reduce relapse rates and subsequent CNS injury due to demyelination[68]. Although current evidence suggests rituximab is effective, and has discontinuation rates lower than is the case for other

DMD's[66], this treatment is not yet approved for the treatment of MS, and is therefore only used off label. This medication is currently approved for the treatment of certain autoimmune disorders, including rheumatoid arthritis, as well as certain types of cancers of the blood, including non-Hodgkin's lymphoma and chronic lymphocytic leukaemia[69].

### 2.2.3. Second Line Treatments

Natalizumab is a monoclonal antibody, which suppresses the entry of leukocytes into the CNS[63, 70]. Phase 3 clinical trials have indicated that patients treated with natalizumab had reduced probability of sustained disability progression, and increased probability of remaining relapse free for 2 years. Treatment with Natalizumab, as is the case for all treatment, is not risk free. In particular, the risk of development of progressive multifocal leukencephalopathy (PML), a rare opportunistic infection of the oligodendrocytes and astrocytes, is increased in patients treated with Natalizumab. Although the risk is increased for patients using natalizumab, the probability of developing the disease remains very low for those in treatment[71]. Fingolimod reduces autoaggressive lymphocytes infiltration into the CNS[63], and thus reduces autoimmune CNS damage .

### 2.2.4. Effect of Treatment on Disease Progression

Disease modifying drugs (DMD's) such as the ones described are thought to aid in delaying the time until the onset of SP MS in patients initially diagnosed with RR MS[72]. The reasons behind why DMD's are able to delay the onset of SP MS are thought to relate to their ability to reduce the inflammatory processes MS patients' experience. This can in turn reduce the extent of CNS injury resulting from a dysfunctional autoimmune response, and delay the neurodegenerative process[73]. The progression of disability has also been shown to be reduced for patients treated with DMD's, again providing evidence for a potential protective effect against CNS injury[74].

### 2.2.5. Treatment in Progressive Subtypes

Most immunomodulatory treatments seem only to be effective in predominantly relapsing subtypes of MS[75]. Until recently, there were no approved treatments for PP MS, with all clinical trials showing no evidence of benefits for patients relative to placebos[76]. The reasons for the lack of effectiveness of conventional treatments for progressive subtypes is incompletely understood. One suggestion is that most conventional treatments target immune system abnormalities in the periphery, which may be only a minor aspect

in the accumulation of damage to nerve tissue during the progressive phase. Effective treatment of the progressive stage may therefore require drugs targeting both peripheral and central immune dysfunction[76]. In March 2017, Ocrelizumab, a monoclonal antibody that targets and depletes CD20+ B-lymphocytes became the first treatment to be approved for PP MS[77] after clinical trial evidence of disease activity reduction for PP MS patients treated with Ocrelizumab relative to placebo[78]. The reasons for its success in clinical trials for PP MS patients are incompletely understood, and has to some degree reignited the debate surrounding whether MS is primarily a T- or B-cell mediated disease[79].

### 2.2.6. Recent developments in MS treatment

More recent developments in MS treatment include treatments such as Cladribine, which is believed to be beneficial for patients with highly active RR MS in particular[80]. Cladribine is a deoxyadenosine analogue which reduces the pro-inflammatory response through depleting lymphocytes[80]. Teriflunomide is another anti-inflammatory medication used in MS, in which the proliferation of rapidly dividing cells, including activated lymphocytes, is inhibited[81]. This inhibition is believed to reduce the extent of the inflammatory response, and thus reduce CNS injury and demyelination in MS patients. Alemtuzumab, a drug approved for patient use in 2013, is a relatively recent development and is currently primarily prescribed in instances where two or more drugs indicated for the treatment of MS have received an inadequate response from the patient[82]. Alemtuzumab depletes the supply of T- and B-cell lymphocytes through antibody-dependent, and complement dependent cytolysis. Repopulation of lymphocytes usually begins weeks later. The therapeutic effects of Alemtuzumab are believed to relate to both lymphocyte depletion and repopulation. During repopulation the proportion of regulatory and memory T-cells increases, and the proportion of naïve T cells is decreased[83]. Proportions for B-cell subtypes is also altered, which is thought to affect the auto-inflammatory response in MS patients. The fact that this treatment option results in prolonged lymphocytopenia means it is only generally considered after other treatments have been shown to be ineffective[84], because this state of immunity leaves patients at increased susceptibility to opportunistic infections.

# 3. PROGNOSIS FOR MS PATIENTS

It is known that life expectancy for MS patients is generally approximately 10 years shorter than life expectancy for the non-MS population, although there have been improvements for MS patients over recent years[85]. Alongside reductions in life expectancy, MS patients are at risk of comorbidity from other diseases, including mental health diagnoses such as depression and anxiety[86]. Hypertension and hyperlipidemia have also been reported as common comorbid diseases. Marrie & Horwitz[87] discuss possible mechanisms which could result in the coexistence of MS and other disorders within the same individual. They discuss the possibility that increased utilization of health services could result in higher prevalence of certain diagnoses due to a form of surveillance bias in which there is more opportunity for individuals with MS to be given diagnoses relative to the non-MS population. Another possible pathway discussed by Marrie & Horwitz is that the risk factors for MS could be common to other diseases. For example, smoking and obesity, both believed to be risk factors for the development of MS, are also risk factors for many other diseases and disorders, including hypertension, and hyperlipidemia.

Alongside common risk factors relating to lifestyle being a possible mechanism for comorbidity in MS patients, there is also evidence to suggest MS patients are at increased risk of developing other autoimmune disorders including asthma, and inflammatory bowel disease. The risk for other diseases such as bipolar disorder, and melanoma are also believed to be increased in MS patients[88]. MS patients have been reported to be more likely to have a family history of autoimmune disorders relative to the general population, providing evidence for a possible genetic susceptibility to other diseases relating to the immune system[89].

MS patients have also been shown to be at increased risk of hospitalization due to infection[90]. The reasons behind why MS patients are more likely to be admitted to hospital with infections could in part relate to surveillance bias due to MS patients having more regular contact with health services, providing increased opportunities for diagnosis, and subsequent hospitalization and treatment. Surveillance bias, however, is unlikely to be the only explanation for the increased risk, given that hospital admission indicates a relatively serious condition unlikely to be diagnosed by chance during scheduled visits. Infection-related mortality is also higher among MS patients[85, 90], again providing evidence of a genuinely increased risk of infection rather than the association

being solely related to surveillance bias. The potential reasons for increased infection susceptibility in MS patients could relate to the effect of immunosuppressive treatments, which could inhibit an immune response to infectious agents[84]. In particular, MS patients on immunosuppressive therapies are at raised risk of opportunistic infections including multifocal leukoencephalopathy[71], although even with a raised risk instances of such severe infections remain rare.

### 3.1 DISEASE PROGRESSION IN MS PATIENTS

The Expanded Disability Status Scale (EDSS) proposed in 1983 is the most commonly used tool to evaluate the severity of neurological impairment among MS patients[91]. In general patients show deterioration over time[92]. The scale considers eight functional parameters: bowel and bladder, brainstem, visual, pyramidal, cerebral, cerebellar, sensory, and 'other'. One section of the scale focuses on functional parameters, and a second section focuses on degrees of mobility for patients. The score can range from 0 (normal) to 8 (maximal impairment). The measure should be considered ordinal, meaning differences between scale steps are not constant[93].

The types of disability found in MS patients can be variable depending on the individual. Fatigue is a particularly commonly reported disabling symptom[94], although the reasons behind why MS patients report fatigue to a greater degree than the non-MS population are unclear. Bol et al. (2010)[94] have investigated possible explanations behind the experience of fatigue reported by MS patients, and considered both a biomedical and a cognitive-behavioural explanation. The effect of fatigue on other aspects of health, including mental health, and physical activity can be substantial, highlighting the importance of studying whether the underlying cause of the fatigue is due to the biological processes involved in the disease (biomedical model) or the patients' interpretation of their condition (cognitive-behavioural model). Bol et al. concluded that both biomedical and cognitive-behavioural factors were involved in the impact of fatigue on other aspects of health and disability, and suggest that the effect of biomedical processes on health are mediated by cognitive-behavioural processes and therefore an integrated approach to treatment should be taken.

Pain is another common symptom of MS, which can contribute substantially to disability progression among patients[95]. Pain can be difficult to objectively measure, which has resulted in widely differing estimates of the extent to which MS patients experience pain

relating to their disease. Higher levels of pain are a risk factor for lower levels of physical activity[96], which can in turn have implications for the development of comorbid conditions such as obesity and diabetes, making pain an important symptom to measure. Neuropathic pain in particular is commonly seen among MS patients, and derives from lesions as a result of injury to the CNS[97]. Trigeminal neuralgia, resulting from damage to the trigeminal nerve which runs through the face, has been found to be common among MS patients, with symptoms of sudden shock-like pain to the side of the face the most typical presentation[98]. Trigeminal and non-trigeminal neuralgia are both types of neuropathic pain usually present due to nerve injury in MS patients. Spasticity, another common MS symptom, can limit joint mobility and decrease muscle flexibility, and ultimately induce pain[99].

Alongside progression in disability over time, some patients diagnosed with RR MS go on to develop SP MS. Scalfari et al (2014)[100] found particular risk factors for entering the SP disease stage were disease duration, male sex, older age at MS diagnosis, and high rate of relapse. The authors stated that onset of SP is the most important determinant of long term prognosis, meaning the prevention of transition from RR to SP MS is of paramount importance, and highlights the strong association between disease stage, and disability progression.

# 4. PREGNANCY OUTCOMES FOR MS PATIENTS

There is little evidence to suggest women with MS are at increased risk of adverse pregnancy outcomes relative to women without MS[101]. The majority of past studies have shown no significant difference in the mean gestational age, or birth weight of babies born to MS mothers[102]. Pregnancy outcomes including rates of miscarriage and malformation also do not appear to be increased in MS patients relative to the general population[103, 104].

During particularly the late stages of pregnancy, the relapse rate for MS patients is typically reduced[105]. In the postpartum period, however, rates of relapse tend to increase to above normal rates for reasons which are incompletely understood[105]. There is limited information available on disease course and pregnancy outcomes for women with progressive subtypes of the disease.

### 4.1. PREGNANCY OUTCOMES AND EXPOSURE TO MS DISEASE MODIFYING DRUGS

Pregnant women are usually excluded from clinical trials, so evidence of the effect of MS DMD's on pregnancy outcomes is largely based on the results of observational studies, and is currently somewhat limited. Past research on the effect of interferon exposure on pregnancy outcomes has been conflicting. Some studies have found an increased risk of outcomes including spontaneous abortion and stillbirth among pregnant women exposed to interferon relative to the general population[106], whereas others indicate no increased risk of adverse pregnancy outcomes[107]. Research undertaken as part of the DMF clinical development program has indicated no increased risk of foetal abnormalities or other adverse pregnancy outcomes relative to the general population when women are exposed to DMF during pregnancy[108]. There is also evidence to show the use of GA during pregnancy is likely to be safe, with no increased risk of congenital abnormalities found for infants prenatally exposed to GA relative to those with no prenatal exposure[109].

Prenatal exposure to second line treatments including natalizumab and fingolimod also appears to be safe[110, 111], however small numbers recruited into past studies mean it is not possible to draw definitive conclusions. Studies into the area of MS treatment during pregnancy have proposed future research into identification and measurement of accurate biomarkers for disease activity and prognosis. This would prove helpful when decisions relating to whether or not to treat at different stages of pregnancy are being made[112]. Currently, the most common approach in Sweden is to cease all MS treatment during gestation in part due to the natural immune suppression which occurs during pregnancy[113]. The guidelines for MS DMD's including interferon state treatment should be discontinued during pregnancy. Further research with populations of MS women are needed in order to gain insight into whether the advice of the guidelines is warranted.

# 5. AIMS OF THE PRESENT STUDY

Past research into prognosis and consequences of living with multiple sclerosis as described in the previous chapters provided the impetus for the current study. The study was separated into four main aims, each of which comprised a separate paper, which considered different stages of the life course for MS patients, and focused on different aspects of the disease and the implications for MS for patients. With topics covering issues relating to pregnancy, symptom management, and life expectancy, the study aimed

to cover the consequences of living with MS over the years from the time many individuals are diagnosed, up until the end of life.

The specific aims of each study were:

1. To consider to what extent MS patients were at increased risk of mortality at any given age relative to individuals without MS, and whether MS patients were at particularly elevated risk of death from a specific cause relative to those without MS. A secondary aim was to assess whether there were changes over time in terms of survival for MS patients, and how this compared to those without MS. Whether there had been changes in cause specific mortality over time for MS patients relative to non-MS comparators was also considered.

2. To assess whether the hypothesised association between MS and pain was evident using pain medication use as a proxy. Whether MS patients were at particularly high risk of neuropathic pain, migraine, or musculoskeletal pain relative to non-MS comparators was a secondary aim.

3. To assess whether the DQB1*0302 genotype is associated with the risk of pain, and whether the risk is modified by the presence or absence of MS given the findings of previous studies.

4. To investigate whether exposure to interferon-beta, a commonly used MS DMD, impacted on the birth measurements of infants prenatally exposed to the treatment, relative to those unexposed to any MS DMD's.

# 6. CONSEQUENCES OF MS THROUGH THE LIFE COURSE

## 6.1. CHILDHOOD AND ADOLESCENCE

Pediatric onset MS (POMS) is rare, with less than 1% of MS patients presenting with symptoms under the age of ten[114]. It has been observed that a higher proportion of POMS patients are diagnosed with the RR subtype of MS relative to the adult-onset MS population, with relapses appearing more frequently in RR MS POMS patients than

would be expected for those diagnosed with RR MS in adulthood[115]. POMS patients are increasingly recognised as having a more inflammatory disease course, which can have consequences for disease progression[114], with this patient group appearing to reach disability at a younger age, and have a poorer long term prognosis than their adult-onset MS counterparts[116]. There have been no large placebo-controlled studies considering whether POMS responds to treatment in the same way as adult-onset MS, and no MS DMD's are currently approved in POMS patients. DMD's prescribed for the adult-onset population are currently also used in POMS patients, however it is plausible, given the more inflammatory disease course, that adult treatments may not be the ideal option for POMS patients.

## 6.2. ADULTHOOD

### 6.2.1. MS and Economic Activity

The majority of MS patients are diagnosed in adulthood, with a mean age of diagnosis of approximately 35 years. One of the most commonly reported and difficult to manage symptoms among MS patients is fatigue, which can impact on capacity to work, and fully engage in daily activities[117]. Despite being one of the most common MS symptoms, fatigue remains a poorly understood research area, however several mechanisms for why this patient group experiences fatigue to a greater degree than the general population have been proposed[118]. These possible pathways have been broadly discussed in terms of primary, and secondary mechanisms. A compelling argument for a possible primary mechanism entails the effect of axonal loss, which can result in compensatory reorganisation, requiring increased brain activity from the patient relative to what would have been required had axonal loss not occurred. This can result in depletion of energy through elevated demand on functioning neural circuits[119]. Secondary mechanisms relate to the possible association of fatigue with disease burden, including increased risk of sleep disorders, depression, and disability among MS patients, outcomes which have been linked to fatigue[118]. In these instances, the comorbidity would likely be acting as a mediator between MS and fatigue. Many MS patients require adapted working environments in order to be economically active, including regular relaxation time and reduced working hours relative to the general population[120]. Pain, another commonly reported symptom of MS patients, has previously been associated with fatigue[121]. Pain can impact on quality of life, including through its impact on fatigue, in a number of

ways, including through interrupting sleep, and increasing the demands of physical mobility[121]. Experiences of pain also reduce the capacity for economic activity in MS patients, particularly if the work is physically demanding, and can be a leading cause of disability among this patient group[122]. The consequences of these experiences of MS symptoms on adult patients can ultimately reduce economic activity, which can impact on income, and other resources.

### 6.2.2. MS and Parenting

In addition to the impact of MS on economic activity, patients are commonly diagnosed at an age when parenting is becoming an important issue for many[123]. This has particularly strong implications for women, due to the issues surrounding treatment decisions during pregnancy, as has been previously discussed. Women comprise two thirds of MS patients, so the majority of those diagnosed will be affected by these issues if they wish to become parents. In addition to the previously mentioned issues relating to pregnancy, MS can impact on parenting itself[124], particularly if the patient experiences a relapse, or has a progressive MS subtype which can mean symptoms are particularly pronounced. Past research has reported children whose parents have MS can feel a greater sense of burden, and felt a higher degree of responsibility relative to the children of healthy parents[125], which can result in behavioural problems[126]. Some studies, however, have concluded that the effect of having a parent with MS on children can be positive. For example, having a parent with MS has been shown to be associated with pro-social behaviour in youth[127], and with increased empathy in children[128]. Whilst there have been studies looking into parenting with an MS diagnosis, it remains an under-researched area, meaning the long term impacts, both positive and negative, of parenting with MS on both the parent and child, remain incompletely understood and further research is warranted[129].

## 6.3. LATER ADULTHOOD, MS, AND DISEASE PROGRESSION

Diagnosis after the age of fifty is considered to be rare[130]. Research into the area of how those with later onset MS are affected is an underreported research area[131], however there are indications that those diagnosed later in life are at substantially increased risk of having a progressive subtype of MS relative to those diagnosed at a younger age[130]. Regardless of age at diagnosis, those aged 50 or over are at increased risk of living in the

progressive disease stages whether it be PP MS or SP MS, which has relevance for quality of life and daily activities for this age group.

For those diagnosed before fifty, one of the main determinants of long term prognosis and disease burden as patients age is whether those initially diagnosed with RR MS transition to SP MS[100, 132]. Research into how this transition can be prevented is therefore of vital importance. Disease course is often unpredictable, and the underlying mechanisms behind why some RR MS patients progress to SP MS and others do not is incompletely understood[133]. The effect of MS DMD's once the progressive phase has been reached is less clear than is the case for RR MS, highlighting the importance of preventing transition to SP MS since treatments cannot necessarily be depended on to alleviate symptoms to the same degree[134].

Current evidence indicates approximately a quarter of MS patients are mature adults over the age of 65, a number likely to rise as life expectancy improves[135]. Living with MS is known to reduce health related quality of life (HRQOL) in this age group, with influence on physical functioning, activities of daily life, and satisfaction with life all factors used in HRQOL measures that have been associated with MS. Past studies have argued that, whilst not a substitute for MS treatment, understanding the factors which reduce HRQOL among older adults with MS is of importance when trying to understand disease perception and how life is affected for this growing patient group[135].

### 6.4. END OF LIFE AND LIFE EXPECTANCY ISSUES

Whilst past research has indicated a 7-14 year reduced life expectancy for MS patients relative to those without MS[136], improved survival for MS patients over time has been observed, and MS survival is beginning to more closely reflect the survival rates seen amongst those without MS[85]. In addition to a shorter average life expectancy, years spent with a disability tends to be higher among MS patients relative to those without MS, and the compression of morbidity seen in the general population[137] is reduced amongst MS patients who tend to live for a longer period of time with a disability over the life course[138]. Recent trends, however, seem to indicate reduced rates of progression from RR to SP MS due at least in part to the development of DMD's[72], with many patients living into old age without acquiring a progressive disease subtype[133]. The reduction in the conversion rate from RR to SP MS could be driving the improvements to the health and

wellbeing of MS patients reaching older age, and ultimately improving both the morbidity and mortality of the MS population over time[139].

# 7. DATA SOURCES FOR MS RESEARCH IN SWEDEN

## SWEDISH REGISTERS

Public administration and health services maintain various electronic records, many of which can be accessed for research through the Swedish registers maintained by the National Board of Health and Welfare (Socialstyrelsen) and Statistics Sweden. All Swedish residents are provided with a unique personal identification number at birth or immigration, which allows for these registers to be linked[140]. The registers discussed in this chapter are relevant for the study of MS patients and were utilised for this thesis.

### 7.1. THE TOTAL POPULATION REGISTER

The total population register began in 1968 and has been maintained since then by Statistics Sweden (SSB)[6]. Information within the register contains inhabitants place of residence, civil status, sex, date of birth, date of death, and date of migration and has done so annually since 1968.

### 7.2. THE NATIONAL PATIENT REGISTER (NPR)

In Sweden, the National Patient Register (NPR) provides data on the diagnoses of Swedish residents, including diagnoses of MS. Data on inpatient visits has been available since 1964, however coverage was not universal until 1987[141]. Since 2001, data has also been available on outpatient visits[142], with coverage improving over time. The register contains information on the International Classification of Disease (ICD) codes for primary diagnoses, alongside information on secondary diagnoses. Date of admission for the in- and out-patient registers, as well as discharge date for the inpatient registers are available. Procedure codes are also included in the register. Whilst coverage for these registers is high, the validity of the data varies depending on the diagnosis. This data is held by the Swedish National Board of Health and Welfare (NBHW), known in Swedish as Socialstyrelsen.

## 7.3. THE PRESCRIBED DRUGS REGISTER (PDR)

Information on prescribed treatment can be found in the Prescribed Drugs Register (PDR). The PDR contains information on the drug prescribed using anatomical therapeutic classification (ATC) codes, on the date of prescription, dispensation, dosage, and brand name of the treatment. This register does not contain information on treatments administered in the hospital setting. Data from this register is available from July 2005. Only drugs collected at the pharmacy through a prescription are found in the PDR, which means treatments which are purchased over the counter, or which are never collected by the patient are not observable using this register. The data is held by NBHW.

## 7.4. THE CAUSES OF DEATH REGISTER

This register began in 1952, with universal coverage achieved in 1961. Since 1961, the register has been annually updated by NBHW, who hold this data[143]. The register contains information on primary and secondary causes of death using ICD codes, and date of death. The data is most reliable for those individuals who died in hospital in terms of both date and cause, with reliability decreasing with the length of time since the last hospital admission.

## 7.5. THE MEDICAL BIRTH REGISTER (MEDISKA FODELSEREGISTRET- MFR)

The Swedish medical birth register (MFR) provides information on all births in Sweden since 1973, and includes data on diagnoses of the mother and infant during and immediately after birth. Information is also collected on pre-existing diagnoses of the mother, smoking status of the mother, and mode of delivery[144]. Birth measurements are also recorded in this register. Data from the MFR can be linked to other register data including the MS register, and NPR using the unique personal identity number issued to all Swedish residents at birth or immigration. The data is held by NBHW

## 7.6. THE SWEDISH MULTIPLE SCLEROSIS REGISTER (MSR)

Since 1996, the Swedish MS quality register, a tool intended to monitor the level of care provided to MS patients in Sweden[145], has provided more detailed information on patient disease status, treatments given, and measures of disability[145]. The register collects information on hospital administered treatments, and estimates of disability using the

expanded disability status scale (EDSS). Relapses are also recorded in this register. However, coverage is not complete and stands at roughly 80% of the prevalent MS population of Sweden[145]. Inclusion in this register requires the consent of the patient. As well as providing more in depth information on MS patients, this resource has a higher positive predictive value (PPV) of MS diagnoses than the patient registers[145]. This register is held at the Karolinska Institute.

### 7.7. THE LONGITUDINAL INTEGRATED DATABASE FOR HEALTH INSURANCE AND LABOUR MARKET STUDIES (LISA)

The longitudinal integrated database for health insurance and labour market studies (Longitudinell integrationsdatabas för sjukförsäkrings, LISA) contains information on education, income, and occupation[146]. LISA was officially initiated in 2003, however Statistics Sweden has been compiling the information included in the registers since 1990 through information provided by the Swedish Social Insurance Agency (Försäkringskassan) which provides sick and disability leave information, the Swedish Public Employment Service (Arbetsförmedlingen) which gives information on employment status, and the Education Register (Utbildningsregistret) providing data on the educational attainment of residents.

### 7.8. THE MIGRATION REGISTER

Whilst this register was not used directly as an outcome or exposure in these MS studies, it was used to identify end of follow-up for emigrees. Information on date of immigration, and emigration are recorded and made available for study by the Swedish Migration Board[6].

## OTHER DATA SOURCES

### 7.9. EIMS, GEMS, AND IMSE

The Epidemiological Investigation into MS (EIMS), Genes and Environment in MS (GEMS) and Immunomodulation and MS Epidemiology (IMSE) studies all began as case-control studies attempting to understand the role of environmental and genetic factors in MS diagnosis and progression[147]. In addition to collecting questionnaire data on lifestyle and exposures, blood samples were also collected from participants, which

allowed for genotyping and study into how genes could influence outcomes for a group of MS patients, and a group of matched comparators.

# 8. METHODS

## 8.1. STUDY DESIGN AND DATA

### 8.1.1. Study 1

Study 1 utilised a classic cohort design, in which all individuals identified as having had a diagnosis of MS either from the patient register, or through the MS register were identified and matched to ten randomly selected non-MS individuals who shared the same year of birth, sex, and county of residence at the time the MS patient was diagnosed. Data from the Total Population Register, Causes of Death Register, NPR, LISA, migration register, and MS register were utilised for this study. All matched comparators had to be alive at the point the MS patient was diagnosed. The matched cohort design uses differently exposed cohorts with shared (matched) characteristics (for example year of birth and sex), and follows up individuals over time to observe whether or not they experience the outcome. In this instance, the outcome was a record of mortality or, for cause specific analysis, a record of a particular cause of death according to the Causes of Death register. The study began in 1968 when the Total Population Register became available for the selection of controls. Whilst some MS diagnosed individuals were identified before 1968, it was not possible to match them to non-MS comparators until this point, so these earlier diagnosed patients were excluded from the study. Data was available until the end of 2012.

### 8.1.2. Study 2

This study made use of the EIMS, GEMS, and IMSE studies[147-150]. These studies were all designed as case-control studies, whereby MS patients were recruited during their visit to a neurologist. If the patient agreed to partake, up to two randomly selected individuals were taken from the Total Population Register matched by sex, year of birth, and place of residence at the point the MS patient was recruited into the study. For the purposes of study 2, intended as a study into how MS patients compare to others in terms of pain, all three studies were combined. Whilst originally designed as a case-control study in which questions on lifestyle and exposures were asked of participants, we used the data as a

cohort study which considered MS as an exposure. For all participants, Swedish register data was collected, and in addition to the EIMS, GEMS, and IMSE data, the PDR, Causes of Death Register, Migration Register, and LISA data were used. Whilst additional data is available in EIMS, GEMS, and IMSE, only register data was used for the purposes of this study. The research question looked at risk of pain amongst MS patients relative to non-MS comparators, and used prescriptions for pain relief as a proxy for a pain diagnosis as the outcome.

### 8.1.3. Study 3

The same data as was used for study 2 was used for this paper, along with additional information collected on genetics. For the collection of genetic data, participants were required to deposit a blood sample. For MS patients, this was undertaken when they visited their neurologist, however the non-MS comparators were required to visit their local health centre (vårdcentralen) for this. Again, the study was used as a cohort study, with exposure considered to be presence or absence of the DQB1*0302 allele. This allele has previously been indicated as associated with pain in patients undergoing inguinal hernia and lumbar disc herniation surgery, and has additionally been implicated in pain expression in murine studies. The outcome was the same as study 2, namely whether medications intended for pain relief were prescribed. MS was considered as a possible effect modifier.

### 8.1.4. Study 4

This study was part of an international collaboration, and included data from Sweden and Finland. All pregnancies to women with MS between 2005-2014 in Sweden were identified through the MS register or NPR, and between the same years in Finland, with MS identified using the Care Register for Health Care within Finland. Data on pregnancy outcomes was obtained from the MBR of the respective country. Birth measurements including birth weight in grams, birth height in cm, and head circumference in cm are recorded in the MFR, and exposure to treatment can be located in the Swedish and Finnish PDR's, with additional information available in Sweden from the MS register. This was also a cohort study, in which women were categorised according to their exposure status, and followed up to ascertain birth measurements for their infants upon delivery.

## 8.2. STATISTICAL METHODS, OUTCOMES, AND EXPOSURES

### 8.2.1. Study 1

The primary analytical method for this study was Cox proportional hazard (PH) models. These models are a commonly used statistical method specifically for analysing time-to-event data[151]. Individuals are followed up from the date they entered the study, in this instance the date the MS patient within the matched group was diagnosed, up until the date of death, date of migration, or the end of the study on 31st December 2012, whichever occurred first. The underlying time scale was attained age, allowing for an interpretation of the hazard ratio (HR) of mortality at any given age for MS patients relative to those without MS. An important assumption of these models is the proportional hazards assumption, which states that the ratio of the hazard for the event for the exposed and unexposed groups is the same over time. In this study, the proportional hazards assumption was tested through interacting the exposure with the underlying time scale, and a stratification variable added which separated time into year of entry if the assumption was violated. The addition of this variable accounted for differing proportional hazards across years. ICD codes were used to identify the primary and secondary causes of death. The specific ICD codes used for cause specific mortality outcomes are displayed in table 1.

**Table 1**- ICD codes used to determine cause of death

**ICD codes**

| | ICD 8 | ICD 9 | ICD 10 |
|---|---|---|---|
| CVD | 39-45 | 39-45 | I0-I9 |
| Respiratory | 460-519 | 460-519 | J0-J9 |
| Infection | 001-139, 680-686 | 001-139, 680-686 | A0-A9, B0-B9, L0 |
| Injury | 800-999 | 800-999 | V0-V9, W0-W9, X0-X9, Y0-Y9 |
| MS | 340 | 340 | G35 |

In addition to considering overall and cause specific mortality, this study aimed to assess whether trends were present for both overall and cause specific deaths, and whether these

trends appeared to differ for MS patients relative to their matched comparators. In order to study this, we divided the time period between 1968-2012 into roughly 10 year periods (1968-1980, 1981-1990, 1991-2000, and 2001-2012). Each time period was analysed separately, with each individual included in the analysis for all the time periods to which they contributed study time.

### 8.2.2. Study 2

Cox PH models were also utilised for this study, which used 1st July 2005 or the date of MS diagnosis for the patient in the matched group as the date of entry, whichever occurred last. The reason for this is the PDR began on 1st July 2005, so outcomes of pain medication dispensation could not be identified before this date. The underlying time scale for this analysis was time since study entry, with follow-up ending on the date of death, date of pain medication prescription, or 31st December 2014, whichever occurred first. The outcome was a binary variable indicating whether or not pain medication had been dispensed during follow-up according to ATC codes recorded in the PDR. Different treatments can be prescribed depending on what the prescribing clinician believes the underlying cause of the pain to be, allowing for identification of specific pain types using pre-specified ATC codes. Pain was categorised into musculoskeletal pain, migraine, and neuropathic pain. The ATC codes used for each outcome are displayed in table 2 below. In addition to considering differences between MS patients and matched comparators, the study also compared the MS cohort members with each other in terms of risk of pain according to age and duration since MS diagnosis.

**Table 2**- Codes used to identify pain outcomes

| **ATC codes** | |
|---|---|
| All pain medication | N02A N02BE M03BB M03BC N02BA N03AX12 N03AX16 N02C N02BG10 N06AA09 N06AA10 |
| Migraine | N02C |
| Musculoskeletal | M03BB M03BC |
| Neuropathic | N03AX12 N03AX16 M02AB N06AA09 N06AA10 |

In addition to model construction, the waiting time distributions were also examined. These methods allow for a visual representation of time from study entry until first prescription, and can therefore provide a rough gauge of the proportion of the cohort who are likely to be prevalent users at study entry, and the proportion likely to be incident users[152]. This is particularly insightful when left truncation is present, as is the case for this study.

### 8.2.3. Study 3

Logistic regression models were used for this study, with genotype considered as the exposure, and pain medication utilisation again considered as the outcome, as a proxy for pain. Such models are commonly used in epidemiological studies with binary outcomes. In this instance, the outcome was the same as study 2, namely whether pain medication had been utilised according to the PDR during follow-up. Neuropathic pain was also considered as an outcome. The primary exposure of interest was genotype, and whether the individuals possessed the DQB1*0302 allele. Additionally, whether zygocity impacted on the risk of pain medication use was assessed, allowing for insight into whether exposure to a higher number of DQB1*0302 alleles impacted on the risk for pain. In order to assess whether the impact of the genotype differed according to MS status, the analysis was stratified according to whether or not the individual had MS, which can provide evidence of whether or not MS acts as an effect modifier for the DQB1*0302 genotype. The outcomes were general pain and neuropathic pain according to ATC code record, and were defined in the same way as study 2 (see table 3 all pain medication and neuropathic section for list of codes).

### 8.2.4. Study 4

This study considered pregnancy outcomes for women with MS according to their exposure to MS disease modifying drugs (DMD's), primarily interferon-beta (IFN-beta). Generalised estimating equations (GEE) were utilised for this study, and birth measurements were considered as continuous variables. Exposure to IFN-beta was classified as a binary variable with a value equal to 1 if the pregnancy was identified as exposed. Pregnancies which were not exposed to any MS DMD's were considered to be unexposed. A sensitivity analysis in which pregnancies exposed to any MS DMD's were

compared to pregnancies unexposed to any MS DMD's was also undertaken. Exposure was identified using ATC codes in the PDR, and through drug names in the MS register. In order to be considered as exposed, the woman needed to have collected a prescription from the pharmacy in the 6 months prior to LMP, under the assumption that the prescription is intended to last 3 months. Exposure therefore begins 3 months prior to LMP. This was the case for both Sweden and Finland. Within Sweden, it was also possible to identify whether treatments had been initiated during the exposure window as recorded in the MS register, in which case the pregnancy was also considered to be exposed. For identification within the MS register, the brand name of the treatment was needed. The ATC codes and treatment brand names are included in table 3.

**Table 3**- ATC codes and drug names used to identify exposure to IFN-beta

| **Treatments used to identify interferon exposure** | |
|---|---|
| **Brand names** | Avonex Plegidry Betaferon Extavia Rebif |
| **ATC codes** | L03AB07 L03AB08 L03AB13 |
| **Treatments used to identify any MSDMD (same as used to identify interferon-beta plus the codes listed below)** | |
| **Brand names** | Copaxone Gilenya IVIG Tecfidera Mitoxantrone Aubagio Lemtrada Tysabri Cladribine |
| **ATC codes** | J06BA02 L01AA01 L01BA01 L04AX03 L01BB04 L01DB07 L01XC04 L03AX13 L04AA13 L04AA23 L04AA27 L04AA31 L04AX01 N07XX09 |

Birth weight in grams, height in cm's and head circumference in cm's as recorded in the MFR were used as separate outcomes. These models allow for consideration of the clustered nature of the data, in which siblings are clustered within the same mother. The GEE approach accounts for the fact individuals within the same cluster are more similar to each other than randomly selected individuals, and adjusts the variance accordingly.

**SIBLING COMPARISON**

For this study, a differently exposed siblings family design approach was also undertaken as a sensitivity analysis. This method utilised cases where, within groups of siblings, at least one of the infants was prenatally exposed to treatment, and at least one infant was not pre-natally exposed to treatment. Sibling comparisons such as this can be considered as a quasi-experimental approach, in which traits other than the exposure of interest are randomised between siblings[153]. Confounding due to for example lifestyle or circumstance of the study subject cannot occur, because subjects cannot make decisions about exposure prior to their own birth. In these instances, all family constant confounders, including those which are unobserved, are controlled for. Confounders which are not family constant, for example maternal age and parity, need to be included in adjusted models when the differently exposed sibling design is used.

# 9. RESULTS

## 9.1. STUDY 1

The study utilised data from 29617 MS patients matched to 296164 non-MS comparators. The study population characteristics are shown in table 4.

**Table 4** Characteristics of MS and non-MS cohorts

| | With MS (%) | Without MS (%) |
|---|---|---|
| Total | 29617 | 296164 |
| Sex | | |
| Female | 19658 (66.4) | 196576 (66.4) |
| Calendar period at study entry | | |
| 1968-1980 | 5197 (17.55) | 51964 (17.55) |
| 1981-1990 | 5224 (17.64) | 52240 (17.64) |
| 1991-2000 | 5777 (19.51) | 57770 (19.51) |
| 2001-2012 | 13419 (45.31) | 134190 (45.31) |
| Age at MS diagnosis/entry | | |
| <18 | 378 (1.3) | 3757 (1.3) |
| 18-40 | 11684 (39.5) | 116931 (39.5) |
| 41-64 | 14016 (47.3) | 139970 (47.3) |
| >=65 | 3539 (12.0) | 35506 (12.0) |
| Follow-up time | | |
| <5 | 7806 (26.4) | 62088 (21.0) |
| 5-10 years | 8451 (28.5) | 75918 (25.6) |
| 10-15 years | 5065 (17.1) | 48252 (16.3) |
| >15 | 8295 (28.0) | 109906 (37.1) |
| Mean (SD) | 12.1 (0.1) | 14.6 (0.2) |
| Educational level* | | |
| Compulsory school or less | 8199 (27.7) | 78810 (26.6) |
| Upper secondary | 11719 (39.6) | 117228 (39.6) |
| Higher education | 8061 (27.2) | 84927 (28.7) |
| No educational data available | 1638 (5.5) | 15199 (5.1) |

* In Sweden compulsory school is 9 years and upper secondary is 3 years.

Within these cohorts, there were 9563 MS deaths, and 59295 non-MS deaths recorded in the Causes of Death register during the study period (1968-2012). The findings identified that at any given age, the HR for mortality for MS patients relative to those without MS was 2.92 (95% CI 2.86-2.99). The largest differences between the cohorts was seen for

respiratory diseases, for which the HR was 5.07 (95% CI 4.87-5.26). Table 5 provides details on overall and cause specific mortality.

**Table 5** Hazard ratios (HR) and 95% confidence intervals (CI) for mortality among MS patients compared with non-MS comparators

| | Event | | | |
|---|---|---|---|---|
| | MS | Non-MS | Unadjusted HR (95% CI) | Adjusted HR (95% CI) |
| All deaths | | | | |
| Total | 9563 | 59295 | 2.72 (2.66-2.78) | 2.92 (2.86-2.99) |
| Sex | | | | |
| Male | 3962 | 26365 | 2.57 (2.48-2.65) | 2.75 (2.65-2.84) |
| Female | 5601 | 32930 | 2.88 (2.80-2.97) | 3.06 (2.97-3.15) |
| Cause of death, underlying or contributing | | | | |
| Cardiovascular | 4193 | 36396 | 2.22 (2.15-2.30) | 2.06 (1.96-2.17) |
| Respiratory | 3322 | 12284 | 4.89 (4.70-5.08) | 5.07 (4.87-5.26) |
| Infections | 440 | 2172 | 3.52 (3.89-3.15) | 3.81 (3.40-4.20) |
| Injuries (inc. Suicides) | 633 | 4842 | 2.06 (1.89-2.24) | 1.72 (1.44-2.06) |
| Cause of death underlying only | | | | |
| Cardiovascular | 2593 | 26813 | 1.90 (1.82-1.98) | 1.85 (1.74-1.97) |
| Respiratory | 605 | 3900 | 3.03 (2.78-3.30) | 3.08 (2.82-3.36) |
| Infections | 135 | 727 | 3.42 (2.84-4.12) | 3.76 (3.12-4.53) |
| Injuries (inc. Suicides) | 280 | 2658 | 1.50 (1.32-1.70) | 1.28 (0.94-1.75) |

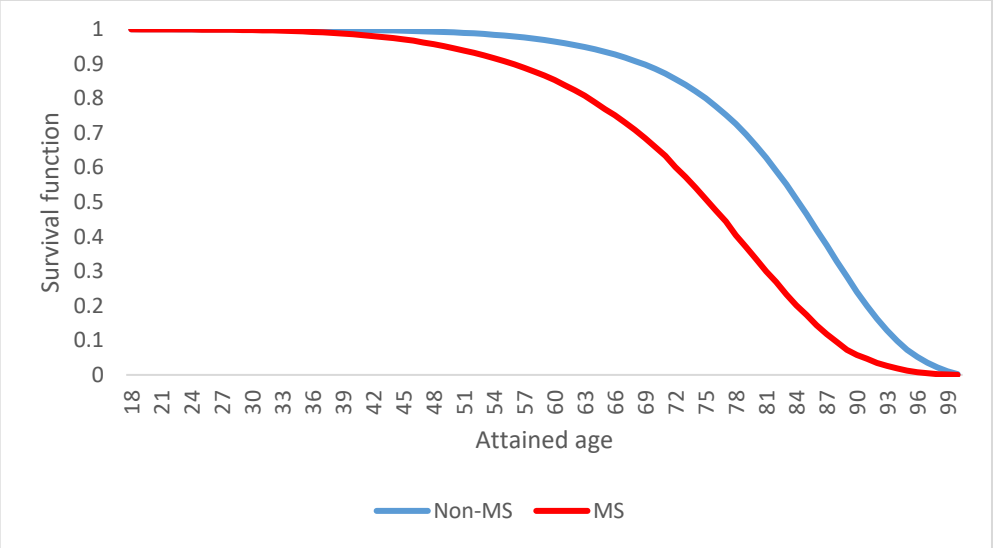
*Adjusted for sex, year of entry, region and highest educational attainment, and stratified by year of entry in the model.

**Underlying or contributing causes % do not add up to 100 because groups are not mutually exclusive

(i.e. 1 individual may be in more than 1 group if they have more than 1 cause of death).

The survival curves for the cohorts are shown in Figure 1.

**Figure 1-** Survival curves for MS and Non-MS cohorts




This study was able to provide strong evidence of improvements in survival over time for both the MS and non-MS cohorts. Importantly, the rate of improvement for MS patients was shown in this study to outpace survival improvements for the matched comparators. This was investigated through an interaction between time period, and exposure. Table 6 below shows the results of this interaction.

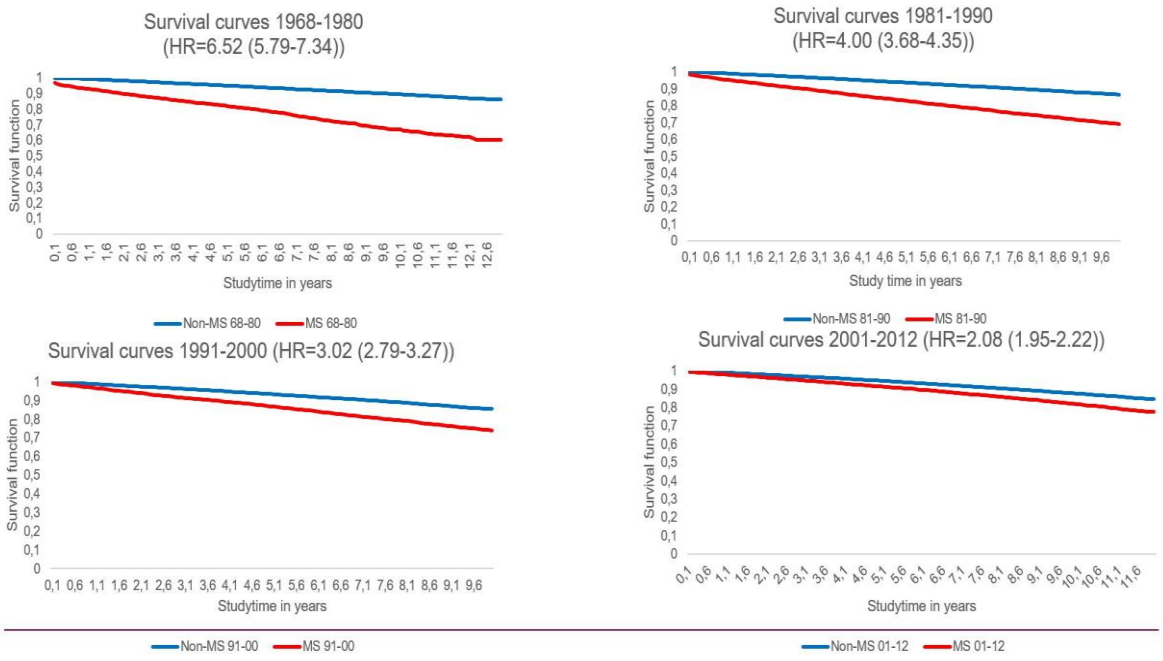
**Table 6** Interaction between time period and MS exposure

| | HR (95% CI) |
|---|---|
| Non-MS | Ref. |
| MS | 2.64 (2.55-2.73) |
| Time period | |
| 1968-1980 | Ref. |
| 1981-1990 | 1.04 (1.00-1.08) |
| 1991-2000 | 0.91 (0.88-0.95) |
| 2001-2012 | 0.79 (0.76-0.82) |
| Time period*MS- | |
| MS*1968-1980 | Ref. |
| MS*1981-1990 | 0.80 (0.53-1.20) |
| MS*1991-2000 | 0.72 (0.48-1.07) |
| MS*2001-2012 | 0.62 (0.42-0.92) |

The interaction shows a larger magnitude movement from the reference time period (1968-1980) in terms of mortality for MS patients, relative to the entire study population. From this, it can be deduced that the MS patients must be improving at a faster rate than their matched comparators, who to some degree are pulling the HR closer to 1 when the entire study population is considered.

The improvements to mortality can also be displayed through survival curves according to time period. Figure 2 shows that whilst there are improvements in survival for both cohorts, there is a clear narrowing of the gap as the survival for MS patients begins to more closely resemble the survival of their matched comparators.

**Figure 2**- Survival curves by time period for the MS and non-MS cohorts




In addition to overall survival improvements, there was a strong improvement in survival for all specific causes of death. Although there were substantial improvements to survival, the largest excess in mortality was still seen for respiratory and infectious diseases in the most recently studied time period (table 7). CVD was shown to be the leading cause of death for both the MS and non-MS cohorts.

**Table 7**- Cause specific mortality trends

| | MS events | Non-MS events | Adjusted HR* |
|---|---|---|---|
| **CVD** | | | |
| 1968-1980 | 483 | 1909 | 6.48 (5.50-7.63) |
| 1981-1990 | 984 | 6434 | 3.22 (2.86-3.63) |
| 1991-2000 | 1147 | 10498 | 2.30 (2.05-2.57) |
| 2001-2012 | 1579 | 17555 | 1.63 (1.48-1.79) |
| **Accidents** | | | |
| 1968-1980 | 33 | 191 | 2.04 (1.41-2.95) |
| 1981-1990 | 92 | 666 | 1.93 (1.56-2.41) |
| 1991-2000 | 170 | 1122 | 2.25 (1.92-2.65) |
| 2001-2012 | 257 | 2254 | 1.63 (1.43-1.86) |
| **Suicides** | | | |
| 1968-1980 | 25 | 70 | 4.06 (2.57-6.41) |
| 1981-1990 | 22 | 168 | 1.70 (1.09-2.66) |
| 1991-2000 | 32 | 197 | 2.14 (1.47-3.11) |
| 2001-2012 | 35 | 315 | 1.38 (0.97-1.96) |
| **Respiratory** | | | |
| 1968-1980 | 354 | 463 | 9.23 (8.03-10.61) |
| 1981-1990 | 707 | 1766 | 5.91 (5.41-6.46) |
| 1991-2000 | 919 | 3403 | 5.12 (4.44-5.90) |
| 2001-2012 | 1342 | 6652 | 2.98 (2.81-3.16) |
| **Infection** | | | |
| 1968-1980 | 8 | 24 | 4.02 (1.80-8.97) |
| 1981-1990 | 16 | 68 | 3.34 (1.93-5.77) |
| 1991-2000 | 66 | 352 | 2.80 (2.15-3.65) |
| 2001-2012 | 333 | 1721 | 2.82 (2.51-3.17) |

*Adjusted for sex, year of birth, place of residence, and highest educational attainment

## 9.2. STUDY 2

This study provided evidence for the hypothesised association between MS and pain risk. The study included 3877 MS patients matched to 4548 non-MS comparators. The cohort characteristics are summarised in table 8 below.

**Table 8**- Characteristics of the cohorts with and without MS

| | With MS, N (%) | Without MS, N |
|---|---|---|
| **Overall** | 3877 (100) | 4548 (100) |
| **Men** | 939 (24.2) | 1080 (23.8) |
| **Women** | 2938 (75.8) | 3468 (76.3) |
| **Age group at MS within matched group diagnosis** | | |
| **<30** | 797 (20.6) | 932 (20.5) |
| **30-39** | 1104 (28.5) | 1293 (28.4) |
| **40-49** | 1086 (28.0) | 1280 (28.1) |
| **50+** | 890 (23.0) | 1043 (22.9) |
| **Educational attainment** | | |
| **Compulsory school or less** | 474 (12.2) | 474 (10.4) |
| **Upper secondary** | 1755 (45.3) | 2015 (44.3) |
| **Further/higher education** | 1645 (42.4) | 2056 (45.2) |
| **No educational data available** | 3 (0.1) | 3 (0.1) |

During the study period, 3082 MS patients, and 2285 non-MS comparators collected prescribed pain medication from the pharmacy according to the PDR.

The study was able to provide evidence for the proposed association between MS and pain through confirming MS patients had an adjusted HR of 2.52 (95% CI 2.38-2.66) for pain medication dispensation relative to their non-MS comparators at any given point during the study period. Neuropathic pain was shown to be the primary reason for this increased pain risk, with an adjusted HR for neuropathic pain medication use of 5.73 (95% CI 5.07-6.47). MS patients also had a significantly increased risk of migraine, however no differences were seen between the cohorts for musculoskeletal pain. Table 9 shows these HR's for adjusted and unadjusted models, along with stratification by sex.

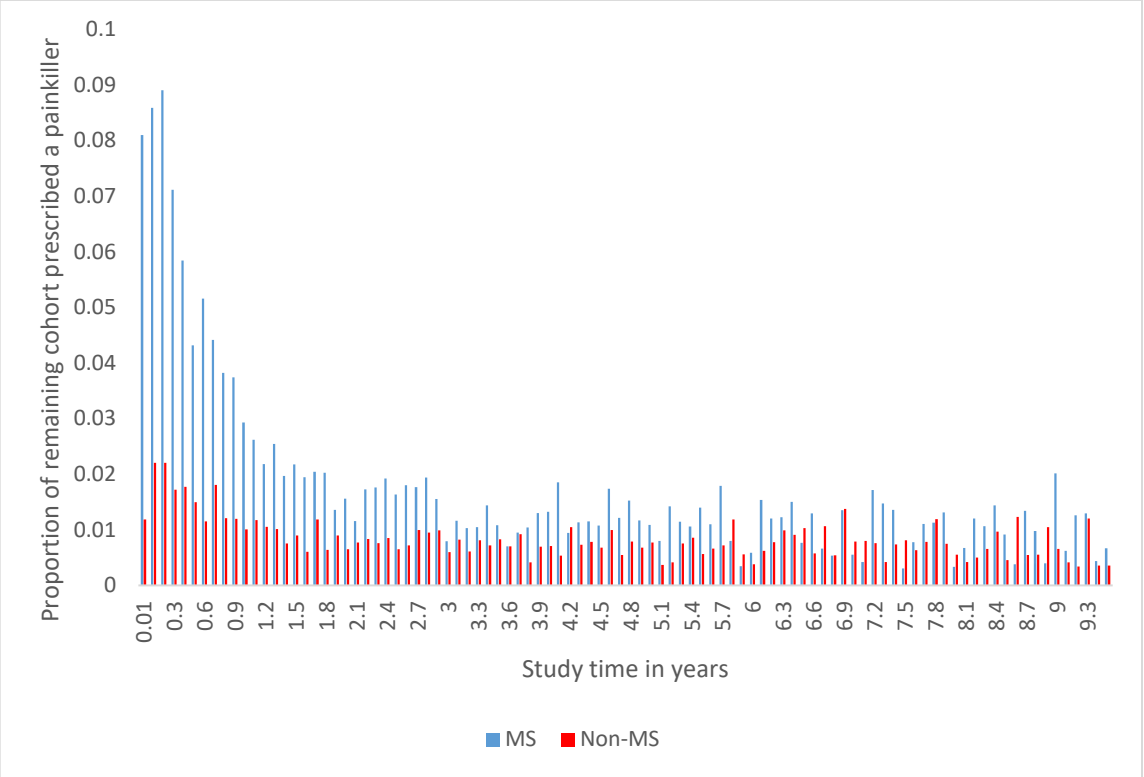
**Table 9**- HRs for pain medication use among patients with MS and non-MS comparators

| | MS | Non-MS | | |
|---|---|---|---|---|
| | **Events (% of cohort)** | **Events (% of cohort)** | **Unadjusted HR** | **Adjusted HR*** |
| **All pain medication** | | | | |
| Overall | 3082 (79.5) | 2285 (59.2) | 2.49 (2.36-2.63) | 2.52 (2.38-2.66) |
| Men | 732 (78.0) | 476 (44.1) | 2.83 (2.52-3.18) | 2.91 (2.58-3.27) |
| Women | 2350 (80.0) | 1809 (52.2) | 2.41 (2.26-2.56) | 2.43 (2.28-2.58) |
| **Neuropathic pain treatment** | | | | |
| Overall | 1326 (34.2) | 325 (7.15) | 5.66 (5.01-6.39) | 5.73 (5.07-6.47) |
| Men | 290 (30.9) | 48 (4.4) | 8.20 (6.04-11.13) | 8.48 (6.24-11.52) |
| Women | 1036 (35.3) | 277 (8.0) | 5.22 (4.57-5.97) | 5.26 (4.61-6.01) |
| **Anti-migraine preparations** | | | | |
| Overall | 250 (6.5) | 247 (5.4) | 1.18 (0.99-1.41) | 1.20 (1.01-1.43) |
| Men | 21 (2.2) | 24 (2.2) | 1.00 (0.56-1.80) | 1.04 (0.58-1.87) |
| Women | 229 (7.8) | 223 (6.4) | 1.20 (1.00-1.45) | 1.23 (1.02-1.48) |
| **Musculoskeletal pain medication** | | | | |
| Overall | 235 (6.1) | 299 (6.6) | 0.90 (0.76-1.07) | 0.90 (0.76-1.06) |
| Men | 49 (5.2) | 54 (5.0) | 1.04 (0.71-1.53) | 1.04 (0.70-1.53) |
| Women | 186 (6.3) | 245 (7.1) | 0.87 (0.72-1.05) | 0.87 /0.71-1.05) |

*Adjusted for sex, region of residence, highest educational attainment, and age at study entry

The waiting time distributions for prescriptions also proved insightful. Their use was particularly helpful in this project due to left truncation because the PDR does not begin until July 2005. Waiting time distributions denote the proportion of newly recorded users of medication at any given point during the study period. A high, left skewed peak indicates a large proportion of users are most likely prevalent at the point the study begins. Figure 3 highlights the disparities in timing of prescription collection for MS patients relative to their non-MS comparators, with the left skewed peak at the beginning of the study time evident for MS patients, but not for the non-MS comparators.

**Figure 3-** Waiting time distributions for MS and non-MS cohorts




In addition to considering whether MS patients were at increased risk of pain, and which pain subtypes appeared to be driving the difference, the study also considered how age and duration since MS diagnosis affected pain medication use. The findings showed that, among individuals who had been diagnosed with MS for the same amount of time, those who were younger were more likely to utilise pain medication (see table 10). The reasons for this are incompletely understood, but could relate to more active disease amongst younger patients.

**Table 10-** Age at study exit adjusting for MS duration for those diagnosed on or after 2005 (MS patients only)

| | N | **Events** | **All pain** |
|---|---|---|---|
| All pain | | | |
| <30 years at study exit | 261 | 223 | 1.87 (1.57-2.22) |
| 30-39 years at study exit | 433 | 344 | 1.40 (1.20-1.63) |
| 40-49 years at study exit | 424 | 322 | 1.24 (1.07-1.45) |
| 50+ years at study exit | 465 | 334 | Ref. |
| Neuropathic pain | | | |
| <30 years at study exit | 153 | 53 | 1.37 (1.01-1.87) |
| 30-39 years at study exit | 423 | 130 | 1.12 (0.89-1.41) |
| 40-49 years at study exit | 438 | 138 | 1.14 (0.91-1.43) |
| 50+ years at study exit | 569 | 168 | Ref. |
| Migraine | | | |
| <30 years at study exit | 133 | 13 | 3.09 (1.56-6.12) |
| 30-39 years at study exit | 384 | 26 | 1.98 (1.13-3.47) |
| 40-49 years at study exit | 435 | 21 | 1.38 (0.76-2.49) |
| 50+ years at study exit | 631 | 23 | Ref. |
| Musculoskeletal pain | | | |
| <30 years at study exit | 129 | 5 | 1.01 (0.39-2.61) |
| 30-39 years at study exit | 378 | 13 | 0.78 (0.41-1.50) |
| 40-49 years at study exit | 447 | 24 | 1.19 (0.69-2.03) |
| 50+ years at study exit | 629 | 30 | Ref. |

*Adjusted for duration since MS diagnosis

**Different N for ages at exit because an event (prescription) indicates age at exit- could be younger for all pain than e.g. migraine

### 9.3. STUDY 3

The same 3877 MS patients and 4548 non-MS comparators utilised for study 2 were also included in this study. Given that the sample and the outcome were the same, the same individuals were identified as having collected a prescription intended to treat pain during the study period (3082 MS patients, and 2285 non-MS comparators). Information on cohort characteristics can be seen in table 9 (unchanged from study 2). Among MS patients, 892 cohort members were identified as being heterozygous for the DQB1*0302

allele, and 32 were identified as being homozygous for the allele. The corresponding numbers for non-MS comparators was 1092 and 79 respectively.

The main finding of the study was the differential effect of allele possession on the risk of pain according to MS status. For MS patients, there was no association between carrying the DQB1*0302 SNP and pain, and there was no effect according to zygocity. The same was not true of the non-MS comparators, for which possession of the gene appeared to increase the risk of pain, particularly for women, and it appeared those homozygous for DQB1*0302 carried the highest risk of pain medication use. Table 11 shows the results when considering the effect of the possession of at least one DQB1*0302 allele, and table 12 shows the results for the zygosity analysis.

**Table 11-** Odds ratios according to presence or absence of the allele

| | PPM | Neuropathic PM |
|---|---|---|
| **Overall** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.09 (0.98-1.21) | 1.07 (0.95-1.22) |
| **Men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.13 (0.92-1.40) | 1.19 (0.90-1.55) |
| **Women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.07 (0.95-1.21) | 1.04 (0.90-1.20) |
| **MS** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.02 (0.85-1.23) | 1.14 (0.97-1.34) |
| **MS men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.06 (0.73-1.54) | 1.09 (0.78-1.51) |
| **MS women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 0.99 (0.80-1.24) | 1.14 (0.94-1.37) |
| **Non-MS** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.18 (1.03-1.35) | 1.19 (0.93-1.54) |
| **Non-MS men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.12 (0.83-1.49) | 1.25 (0.63-2.46) |
| **Non-MS women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ or +/+ | 1.21 (1.03-1.41) | 1.20 (0.91-1.59) |

*Adjusted for PCA's, sex, year of birth, and region of residence, and highest educational attainment

**Table 12-** Odds ratios according to number of alleles possessed

| | PPM | Neuropathic PM |
|---|---|---|
| **Overall** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.08 (0.97-1.21) | 1.09 (0.95-1.23) |
| DQB1*0302 +/+ | 1.17 (0.78-1.75) | 0.87 (0.53-1.45) |
| **Men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.16 (0.93-1.44) | 1.22 (0.93-1.61) |
| DQB1*0302 +/+ | 0.82 (0.41-1.67) | 0.69 (0.24-2.04) |
| **Women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.06 (0.93-1.20) | 1.05 (0.90-1.21) |
| DQB1*0302 +/+ | 1.36 (0.83-2.25) | 0.94 (0.53-1.68) |
| **MS** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.05 (0.86-1.27) | 1.14 (0.97-1.34) |
| DQB1*0302 +/+ | 0.60 (0.28-1.31) | 1.18 (0.56-2.50) |
| **MS men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.12 (0.76-1.64) | 1.10 (0.78-1.54) |
| DQB1*0302 +/+ | 0.44 (0.12-1.59) | 0.88 (0.22-3.52) |
| **MS women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.01 (0.81-1.26) | 1.13 (0.94-1.36) |
| DQB1*0302 +/+ | 0.67 (0.25-1.79) | 1.34 (0.54-3.31) |
| **Non-MS** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.14 (0.99-1.31) | 1.17 (0.90-1.53) |
| DQB1*0302 +/+ | 1.97 (1.22-3.18) | 1.49 (0.69-3.22) |
| **Non-MS men** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.09 (0.81-1.48) | 1.23 (0.61-2.48) |
| DQB1*0302 +/+ | 1.41 (0.59-3.39) | 1.39 (0.17-11.41) |
| **Non-MS women** | | |
| DQB1*0302 -/- | Ref. | Ref. |
| DQB1*0302 -/+ | 1.16 (0.99-1.36) | 1.17 (0.88-1.56) |
| DQB1*0302 +/+ | 2.26 (1.25-4.06) | 1.62 (0.70-3.71) |

*Adjusted for PCA's, sex, year of birth, and region of residence, and highest educational attainment

An interesting aspect of the study was its reflection of the results of past murine models, which indicated possession of the genotype led to increased pain-like behaviour among

rats when considering peripheral nerve injury, however the same results were not found when considering spinal cord injury, which more closely mimics the injury that demyelination causes among MS patients. The fact that MS patients may be at increased risk of CNS rather than peripheral nerve injury could partly account for the lack of significance among MS patients when considering an association between the DQB1*0302 allele and pain medication use.

### 9.4. STUDY 4

The study made use of 1246 pregnancies in Sweden and 563 pregnancies in Finland for which data was available from the corresponding country's MFR on birth weight, height and head circumference. Within Sweden, 411 pregnancies were identified as exposed, and in Finland 232 pregnancies were identified as exposed to IFN-beta. Details of the pregnancy cohort characteristics are shown in table 13 below. The unexposed group consisted of pregnancies unexposed to any MS DMD's.

**Table 13**- Cohort characteristics

| | **Sweden** | | | |
|---|---|---|---|---|
| | **All** | | **Differently exposed siblings** | |
| | **Exposed** | **Unexposed** | **Exposed** | **Unexposed** |
| **Number of pregnancies** | 411 | 835 | 50 | 51 |
| **Infant Sex** | | | | |
| **Male (%)** | 207 (50.4) | 441 (52.8) | 24 (48.0) | 26 (51.0) |
| **Female (%)** | 204 (49.6) | 394 (47.2) | 25 (52.0) | 25 (49.0) |
| **Mean (SE) maternal age, years** | 31.3 (0.2) | 32.3 (0.2) | 31.0 (0.6) | 30.9 (0.5) |
| **Maternal education** | | | | |
| **Compulsory school or less (%)** | 20 (4.9) | 56 (6.7) | 2 (4.0) | 2 (3.9) |
| **Upper secondary (%)** | 113 (27.5) | 242 (30.0) | 15 (30.0) | 17 (33.3) |
| **Higher education (%)** | 277 (67.4) | 534 (64.0) | 33 (66.0) | 32 (62.8) |
| **Missing data (%)** | 1 (0.2) | 3 (0.4) | 0 (0) | 0 (0) |
| **Smoking status** | | | | |
| **Smoker (%)** | 18 (4.4) | 54 (6.5) | 45 (90.0) | 47 (92.2) |
| **Nonsmoker (%)** | 378 (92.0) | 740 (88.6) | 3 (6.0) | 0 (0) |
| **Not known (%)** | 15 (3.7) | 41 (4.9) | 2 (4.0) | 4 (7.8) |
| | **Finland** | | | |
| | **All** | | **Differently exposed siblings** | |
| | **Exposed** | **Unexposed** | **Exposed** | **Unexposed** |
| **Number of pregnancies** | 232 | 331 | 41 | 42 |
| **Infant Sex** | | | | |
| **Male** | 117 (50.4) | 171 (51.7) | 18 (43.9) | 18 (43.9) |
| **Female** | 115 (49.6) | 160 (48.3) | 23 (56.1) | 24 (57.1) |
| **Mean (SE) maternal age, years** | 30.0 (4.2) | 30.6 (4.5) | 30.0 (4.2) | 30.6 (4.5) |
| **Maternal education** | Not available | Not available | Not available | Not available |
| **Smoking status** | | | | |
| **Smoker (%)** | 33 (14.2) | 49 (14.8) | 2 (4.9) | 3 (7.1) |
| **Nonsmoker (%)** | 195 (84.1) | 277 (83.7) | 39 (95.1) | 39 (92.9) |
| **Not known (%)** | 4 (1.7) | 5 (1.5) | 0 (0) | 0 (0) |

Mean measurements for gestational age, and birth weight, height, and head circumference were similar across country and exposure status. Details are shown in table 14 below.

**Table 14-** Mean measurements for birth outcomes

| | **All** | | **Differently exposed siblings** | |
|---|---|---|---|---|
| | **Exposed** | **Unexposed** | **Exposed** | **Unexposed** |
| | **Sweden** | | | |
| **Number of pregnancies** | 411 | 835 | 50 | 51 |
| **Mean (SE) Gestational age, weeks,** | 39.7 (0.1) | 39.5 (0.1) | 40.0 (0.2) | 39.2 (0.3) |
| **Mean (SE) Birth weight, grams** | 3465.9 (27.7) | 3414.8 (19.4) | 3475.5 (66.3) | 3346.6 (81.7) |
| **Mean (SE) Birth height, cm's** | 50.1 (0.1) | 50.0 (0.1) | 50.3 (0.4) | 49.7 (0.4) |
| **Mean (SE) Head circumference, cm** | 35.0 (0.1) | 35.0 (0.1) | 35.0 (0.2) | 34.7 (0.3) |
| | **Finland** | | | |
| **Number of pregnancies** | 232 | 331 | 41 | 42 |
| **Mean (SE) Gestational age, weeks,** | 39.4 (2.4) | 39.5 (1.9) | 39.5 (2.9) | 40.0 (1.2) |
| **Mean (SE) Birth weight, grams** | 3357.5 (628.3) | 3410.4 (541.0) | 3306.6 (649.2) | 3508.4 (441.7) |
| **Mean (SE) Birth height, cm's** | 49.5 (3.1) | 49.6 (2.5) | 49.2 (3.8) | 49.9 (1.9) |
| **Mean (SE) Head circumference, cm** | 34.5 (2.2) | 34.8 (1.7) | 34.4 (2.6) | 35.0 (1.4) |

The findings of the GEE's showed there were no significant differences in terms of birth measurements for infants prenatally exposed to IFN-beta relative to those unexposed. Table 15 shows the adjusted beta estimates for the mean birth measurements for birth weight, height, and head circumference for Sweden and Finland. Gestational age, sex of the newborn, smoking status of the mother, and maternal age at LMP are included as covariates. Results are displayed overall, and for differently exposed siblings.

**Table 15**- Adjusted GEEs for birth measurements

| | Adjusted* | | | | | |
|---|---|---|---|---|---|---|
| | **Weight** | **P-value** | **Height** | **P-value** | **Head circumference** | **P-value** |
| **Sweden** | | | | | | |
| **Overall** | 27.8 (20.1) | 0.34 | 0.01 (0.1) | 0.95 | 0.14 (0.1) | 0.13 |
| **Differently exposed siblings** | -21.6 (77.1) | 0.78 | -0.10 (0.4) | 0.78 | -0.05 (0.3) | 0.85 |
| **Finland** | | | | | | |
| **Overall** | -50.3 (45.1) | 0.27 | -0.02 (0.2) | 0.92 | -0.21 (0.2) | 0.15 |
| **Differently exposed siblings** | -83.6 (79.8) | 0.30 | 0.07 (0.4) | 0.85 | -0.008 (0.3) | 0.98 |

*Adjusted for gestational age, sex of the newborn, smoking status of the mother, and maternal age at LMP

An additional sensitivity analysis which used an exposure window beginning at LMP up until the end of pregnancy was undertaken which showed no significant differences in birth measurements between those exposed to IFN-beta, and those unexposed to any MS DMD's during the same time frame.

A mixed effects model in which the intercept was allowed to vary according to maternal ID highlighted that a large proportion of the variance is derived from differences within sibling clusters, implying siblings are not homogenous for their birth measurements. The adjusted beta again shows the effect of exposure to IFN-beta relative to those unexposed to any MS DMD's. Table 16 shows the results of the mixed effects models. The intraclass correlations demonstrated that overall, 46.4% of the variance for birth weight was explained by differences between rather than within clusters, with the corresponding numbers being 31.9% and 36.8% for birth height and head circumference respectively. For differently exposed siblings, power was greatly reduced, making it difficult to draw conclusions. It suggests the effect of clustering is not radically reducing the variance, because observations within the same cluster are not necessarily very similar.

**Table 16**- Mixed effects model with random intercept for maternal ID

| | Adjusted Beta (SE)* | P-value | Intraclass correlation (% (95% CI)) |
|---|---|---|---|
| **Overall** | | | |
| **Weight** | 27.5 (27.1) | 0.310 | 46.4 (37.4-55.8) |
| **Height** | 0.001 (0.1) | 0.994 | 31.9 (22.9-42.4) |
| **Head circumference** | 0.12 (0.1) | 0.178 | 36.8 (27.5-47.2) |
| **Differently exposed siblings** | | | |
| **Weight** | -24.0 (72.4) | 0.740 | 31.3 (10.8-63.1) |
| **Height** | -0.15 (0.06) | 0.644 | 55.0 (33.9-74.5) |
| **Head circumference** | -0.11 (0.26) | 0.667 | 18.9 (3.8-58.0) |

*Adjusted for gestational age, sex of the newborn, smoking status of the mother, and maternal age at LMP

The lack of effect of prenatal exposure to IFN-beta could be attributed to its pharmacokinetic qualities. Maternal and foetal blood is separated by the placental barrier, which is semi-permeable. In order for substances to pass from maternal to foetal blood, it must have a low molecular weight (between 600 and 800 Dalton[154]). The possibility that IFN-beta could permeate into foetal blood is therefore unlikely, because it is classified as a polypeptide with a molecular weight of 22kDa for IFN-beta 1a and 18.5kDA for IFN-beta 1b[155], which is too large to permeate the placental barrier. This demonstrates that the lack of effect of IFN-beta on birth measurements is biologically plausible.

# 10. METHODOLOGICAL CONSIDERATIONS

Caveats to each of the included studies should be considered. Limitations to the outcome, exposure, study design and analytic method can all influence results, so should be considered when drawing conclusions.

## 10.1. STUDY 1

Within the Causes of Death Register, it is known that the most accurate records of death in terms of both date and cause are for those who died in hospital. For those who did not, the longer ago the last hospital visit, the less accurate the death record tends to be[143]. In addition, the definition of underlying cause or causes of death is a complex process, and can be subjective. Clinicians are required to separate out conditions which contributed to the death from any other existing conditions the individual may have had which did not contribute to death. In addition, the conditions which directly lead to death should be

separated from those which contributed to the outcome, but did not directly lead to it (e.g. for a suicide relating to depression, the method used should be listed as the primary cause of death, with depression listed as a contributory cause). The quality of the entire death register has not been checked beyond the year 1995[156]. More recent validation of the data would therefore be beneficial.

### 10.2. STUDY 2

The main limitation with study 2 is the use of pain medication utilization as a proxy for experiencing pain. Some medications, such as pregabalin and gabapentin are also used as anti-epileptic medications, and amitriptyline and nortriptyline can be used to treat depression. For this reason, for study 2 a sensitivity analysis was conducted which excluded individuals identified as having had a diagnosis of epilepsy or depression in order to ensure consistency of results. However, if treatments are prescribed for a condition other than pain, this will detract from the specificity of the outcome. Conversely, it is not possible to identify those with pain who do not dispense medication at the pharmacy, which impacts on the sensitivity of the outcome. Another issue with this paper is the left truncation due to the PDR beginning in July 2005. It is not possible to identify the outcome before this date, meaning some individuals will have been living with MS for many years before study entry. It was therefore not possible to consider only incident users of pain medication, which would have provided stronger evidence of whether being exposed to MS results in higher rates of pain medication utilization. The waiting time distributions aided in giving some insight into prevalent users, and therefore could be considered a rough gauge for the proportion of each cohort using pain medication prior to the beginning of the study, however it is not possible to entirely separate prevalent from incident users.

### 10.3. STUDY 3

The outcome for this study was the same as study 2, so the same limitations to the outcome apply. In addition, another important consideration for this study is the issue of pleiotropy, which has implications for all genetic studies. Pleiotrophy is the process by which one gene influences two or more traits which may be seemingly unrelated[157], which can make distinguishing between a direct biological effect, and a mediating effect difficult. In addition, complex traits such as experiences and perceptions of pain tend not

to be the result of the characteristics of one particular SNP, rather it is likely they are the result of many genes spread throughout the genome[158]. This means that isolating one SNP for study could be limiting, because it may be the interaction of many SNPs that is ultimately affecting the phenotype. By studying one SNP, it is not possible to gain insight into whether the SNP itself, or other genotypes which tend to occur together with DQB1*0302 are the primary influencers of pain phenotype, or whether there is an interaction effect.

### 10.4. STUDY 4

For study 4, only infants for which a birth weight, height, and head circumference was present were included. All three measurements needed to be available in order to ensure the same infants were being studied throughout. The limitation to this is that bias can be introduced if missingness is differential by exposure status. Within the Swedish data, 25% of the unexposed and 17% of the exposed pregnancies had one or more missing birth measurement resulting in exclusion. The distribution of missingness cannot, however, give an indication as to whether missingness is differential, and bias can still be present even if the proportion missing is the same across two cohorts. In addition, register data is collected for administrative rather than research purposes, meaning there are a limited number of variables available. Therefore, it was not possible to adjust for potential variables of interest such as diet and physical activity, which could be associated (either directly or indirectly) to the decision to continue treatment during pregnancy, and to birth measurements.

Methodological aspects of the differently exposed sibling design should also be discussed. There are three principle assumptions which should be upheld in order for the method to provide results which are generalizable and accurate. The first is exposure, outcome and other covariates must be accurately measured. This is because within-cluster estimates are more severely influenced by non-shared confounders than conventional estimates which assume independence between observations[159]. The second assumption is non-contagion, in which the outcome or exposure of one sibling should not have the ability to affect the outcome or exposure of another sibling[160, 161]. This would mean the birth measurements of one sibling should not have the potential to influence the exposure status of another sibling. The third assumption is that differently exposed siblings are reflective of the general population[160], meaning differently exposed siblings

should not differ in some fundamental way and should be representative of all infants[153], including singletons, and those who are not differently exposed but are part of a sibling group.

These assumptions should be considered and their plausibility assessed in order to avoid inaccurate interpretation of results. With regards to accurate recording of the exposure and other covariates, it seems probable any variations in such accuracy is likely to be at random, and should not majorly bias our results. The other two assumptions may prove more problematic, however. The decision to either continue with or cease treatment for one pregnancy could reasonably sway the decision made in future pregnancies. For example, if an infant is born with a complication (for example abnormal birth measurements, the outcome in this study) and the mother continued treatment during pregnancy, it may result in increased likelihood she decides against treatment during a later pregnancy. This mechanism could work in a number of ways, and a healthy birth for an exposed infant may also reassure the mother treatment is safe, and result in her decision to use it in future pregnancies, meaning her offspring are not differently exposed. This issue leads on to the possible violation of the third assumption, which is that differently exposed siblings are reflective of the general population. This may well not be the case, and mothers who do have differently exposed offspring may make treatment decisions for a variety of reasons, and ultimately the differently exposed sibling population may be fairly particular. This would reduce the external validity of the results.

# 11. ETHICAL CONSIDERATIONS

This chapter will consider the ethical implications of register data use and particular themes relevant to each paper. These considerations will be discussed through the lens of each of the four sections of principlism[162].

The four principles included in the idea of principlism are doing good, avoiding harm, autonomy and justice. They were compiled in response to the syphilis study undertaken in Alabama from 1932-1972[163]. This study used African American subjects known to their clinicians to have syphilis, but were left unaware of their diagnosis, had readily available treatments withheld from them, and were subjected to what they believed were treatments, which were in reality research measurements.

Investigating whether MS patients are at increased risk of particular outcomes, including death, could correspond with the first principle of doing good, because areas where improvements could be made can be highlighted. An alternative perspective is the idea that distress may be caused were patients made aware of the findings of these studies, perhaps in particular the increased risk of death. The ethical standing of this paper based on this principle would therefore depend on the cost-benefit relationship of improvements measured against harm caused by potential distress. In my view, the potential good which could come from the papers outweighs the risk of harm through potential patient distress, but means any reporting of the results particularly to patient groups should be handled with care.

The second principle on avoiding harm is relatively easy to address in relation to register based studies. The life events of patients and other subjects are not affected by inclusion in such studies, and in fact for most register based studies (including the mortality and pregnancy outcome papers in this thesis), individuals are not aware they are part of a study. For papers 2 and 3 in which subjects were enrolled, harm is avoided if any medical procedures are carried out by trained medical professionals, as indeed they were. Additionally, harm in the form of leaked personal data is safeguarded by ensuring all data is kept on a secure server, and is not accessed outside the university setting

For the studies included in this thesis, the most contentious principle is perhaps that of autonomy. Emphasis has been placed on the importance of autonomy and informed consent when using human subjects in research since the Nuremburg trials which occurred after the close of World War II, and came in response to experimentation undertaken without individual consent in concentration camps[164]. Informed consent is not gained for subjects for two out of the four studies which are entirely constructed using register data, which means the issue of autonomy and informed consent should be discussed. The fact that informed consent is not gained could be mitigated if real benefit can be gleaned from these studies. Therefore, the ethical standing of these papers could depend on the extent to which they prove beneficial to patients in the future.

The final principle is justice, which attempts to ensure that vulnerable groups are not unfairly subjected to scientific scrutiny without the potential for real benefits within their group being possible, or the benefits of the findings only being present in other groups[165]. With regard to this thesis, all studies consider MS patients, and are designed with

potential benefits for MS patients in mind. It seems unlikely that the possible health benefits which could come from the papers in this thesis would only be present in other groups, therefore it could be argued that the principle of justice is upheld in this thesis.

A final ethical consideration which is relevant for this thesis outside the framework of principlism is that of conflict of interest, and the influence pharmaceutical companies may have over the papers[166]. For all papers, the funding sources and conflict of interest has been explicitly described and addressed. Furthermore, the pharmaceutical companies were limited in the extent to which they were allowed to influence the content and distribution of these studies. Whilst conflict of interest is clearly described in each paper, it does not entirely remove the implications and is worth contemplating when considering the ethical implications of the thesis.

# 12. CONCLUSIONS

MS is one of the most common non-traumatic causes of disability among young adults in many countries, yet much remains unclear about the consequences of living with the disease. The papers which are included in this thesis aim to add to the evidence base on the prognosis for this group in terms of their mortality, their experience of pain, and pregnancy outcomes which considers the effect of treatments. These studies consider different aspects of the disease, and cover topics ranging from those of concern at the mean age of diagnosis, namely how pregnancy can be affected by treatment, up until the end of life and how life expectancy can be affected by the presence of MS.

Alongside overarching conclusions on consequences of living with MS for patients in Sweden, the thesis also reached more specific conclusions particular to the aims of each constituent paper. Survival time was shown to be reduced among MS patients, however major improvements over the past 40 years were identified. These improvements were evident for cause specific mortality, as well as overall mortality, and suggest the mortality trends of MS patients are beginning to more closely resemble those seen in the general population. The hypothesised increased risk of pain for MS patients was found to be evident using our data, with neuropathic pain in particular driving the increased risk of pain for MS patients. The expression of pain according to DQB1*0302 allele possession was found to be differential according to MS status, a finding hypothesised due to previous associations reported from murine studies. These studies highlighted CNS pain

was differentially influenced by this gene when compared to peripheral pain. Finally, the thesis concluded that exposure to interferon-beta during pregnancy did not seem to be associated with intrauterine growth. This finding is in line with the majority of past studies into the effect of pre-natal interferon-beta exposure on birth outcomes including birth measurements, which have shown null findings when comparing outcomes for those pre-natally exposed to treatment relative to those pre-natally unexposed.

# 13. FUTURE PERSPECTIVES

This thesis has attempted to gain a deeper understanding of the consequences of living with MS for a wide group of MS patients. Whilst the project has provided evidence for particular processes and associations, further work into particular areas would benefit the field of MS research. This could give further insight into how patients are affected both at specific times during their life, for example during pregnancy, and overall, providing information on issues such as symptom experiences and life expectancy.

The extent to which life expectancy is affected by diagnosis with MS has comprised part of this project, however whether this differs according to various factors including EDSS score, age at diagnosis, or disease course (i.e. whether the patient is RR, SP, PP, PR) would provide valuable insight into how more specific groups of MS patients are affected. Additionally, a clear trend was observed in this study which highlighted substantial improvements to survival for MS patients. Whether the gap between those with and without MS continues to narrow in the future would be informative, and help monitor how survival in these patients is progressing.

The experience of pain was here identified using the PDR, allowing for an objective approach rather than being dependent on recall bias. Whilst there are advantages to this, there are drawbacks, such as lacking information on more specifically how quality of life and daily physical and mental functioning are affected. Data which specifically asks participants about pain, and how they are affected could be insightful when for example considering how to retain MS patients in the work force, or how best to adapt to their needs when prescribing certain treatments or physical therapies. Open ended, or qualitative studies, may add more nuance and allow for a more personalised insight into how patients are affected by this particular symptom.

The effect of genotype on disease course and various phenotypes such as pain is a rapidly growing area of interest. This thesis was only able to examine one previously identified single nucleotide polymorphism (SNP) of interest, with many others still to be investigated. The interaction between commonly occurring groups of alleles and pain would therefore be of interest. In addition, our study showed no increased risk of pain amongst MS patients with the DQB1*0302 genotype. Whether there are any particular genotypes which do increase the risk of pain amongst MS patients, in particular those found on the HLA complex, would therefore be of interest.

Whether or not it is safe to continue with treatment during pregnancy is a difficult to study area. The study included in this project indicated there were no implications for birth measurements when women were exposed to IFN-beta during pregnancy. Guidelines tend to act on the side of caution, and will generally state treatments should cease during pregnancy, which could be problematic if the mother experiences symptoms and would ideally be able to continue with treatments. Observational studies such as this one for the other most commonly used MS DMD's would go some way for providing specific guidelines for different MS DMD's, and which treatments would be safest if a woman wishes to continue with treatment after conception.

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